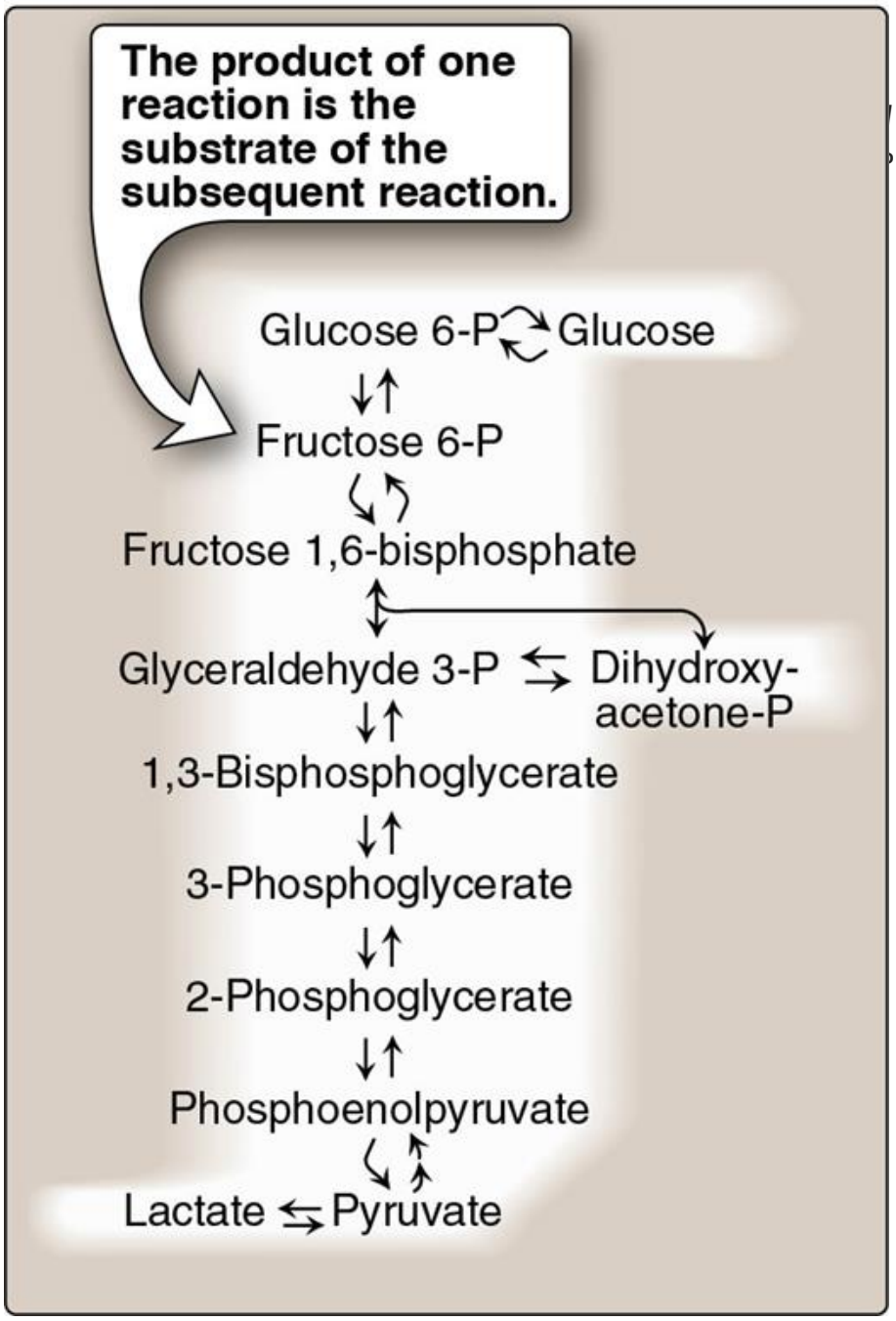
8: Introduction to Metabolism and Glycolysis

Metabolism Overview

In Chapter 5, individual enzyme reactions were analyzed to explain the mechanisms of catalysis. However, in cells, these reactions rarely occur in isolation. Instead, they are organized into multistep sequences called pathways, such as that of glycolysis (Fig. 8.1). In a pathway, the product of one reaction serves as the substrate of the subsequent reaction. Most pathways can be classified as either **catabolic** (degradative) or **anabolic** (synthetic). Catabolic pathways break down complex molecules, such as proteins, polysaccharides, and lipids, to a few simple molecules (e.g., carbon dioxide, ammonia, and water). Anabolic pathways form complex end products from simple precursors, for example, the synthesis of the polysaccharide glycogen from glucose. Different pathways can intersect, forming an integrated and purposeful network of chemical reactions. Metabolism is the sum of all the chemical changes occurring in a cell, a tissue, or the body. Metabolites are intermediate products of metabolism. The next several chapters focus on the central metabolic pathways that are involved in synthesizing and degrading carbohydrates, lipids, and amino acids.

FIGURE 8.1

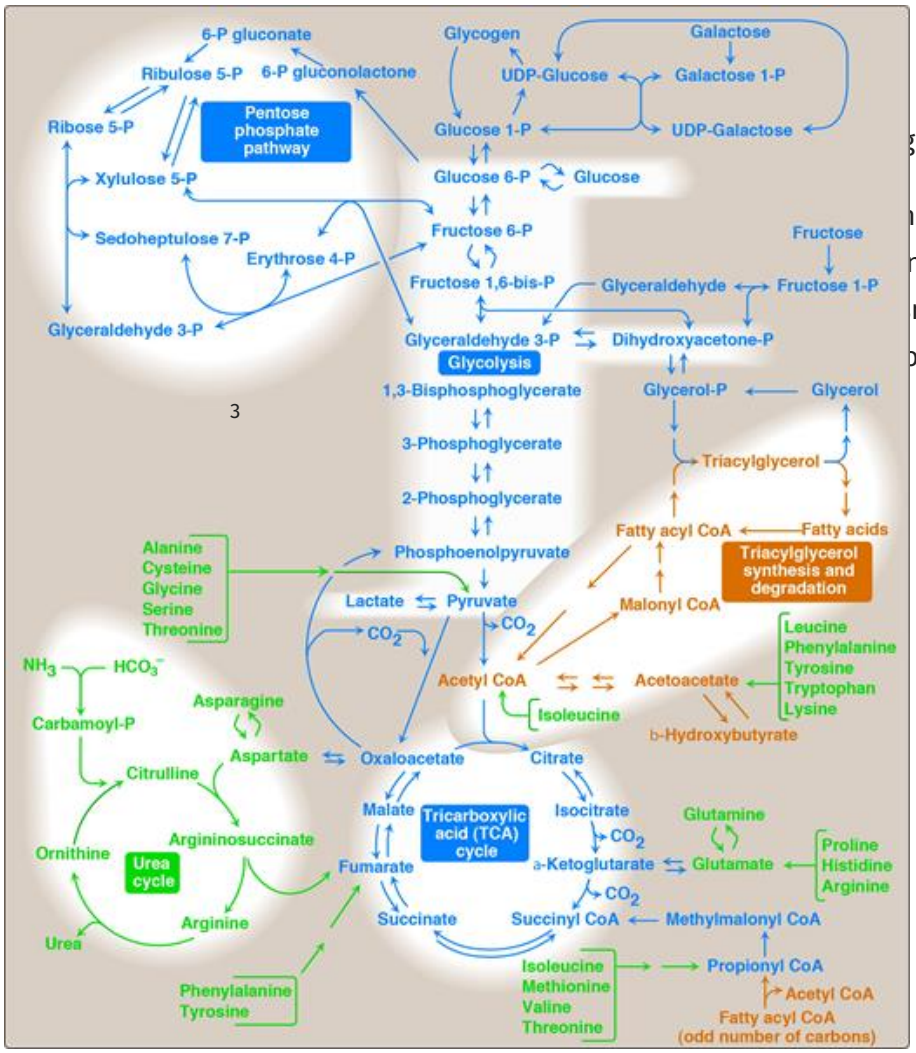


reaction arrows ( ) indicate
= phosphate.

Metabolic map

Metabolism is best understood by examining its component pathways. Each pathway is composed of multienzyme sequences, and each enzyme, in turn, may exhibit important catalytic or regulatory features. A metabolic map containing the important central pathways of energy metabolism is presented in Figure 8.2. This "big picture" view of metabolism is useful in tracing connections between pathways, visualizing the purposeful movement of metabolites and depicting the effect on the flow of intermediates if a pathway is inhibited or blocked, for example, by a drug or an inherited deficiency of an enzyme. Throughout the next three
metal

FIGURE 8.2



ghted. *Curved reaction arrows* ( ) zymes. The *straight arrows* ( ) nzyme. *Blue text* = intermediates of m; *green text* = intermediates of oenzyme A; CO_2 = carbon dioxide; HCO_3^-

Catabolic pathways

Catabolic reactions serve to capture chemical energy in the form of ATP from the degradation of energy-rich fuel molecules. ATP generation by degradation of complex molecules occurs in three stages, as shown in Figure 8.3. (Note: Catabolic pathways are typically oxidative and require oxidized coenzymes such as nicotinamide adenine dinucleotide [NAD^+].) Catabolism also allows molecules in the diet or nutrient molecules stored in cells, to be converted into basic building blocks needed for the synthesis of complex molecules. Catabolism, then, is described as a convergent process in which a wide variety of molecules are transformed into a few common end products.

Hydrolysis of complex molecules

In the first stage, complex molecules are broken down into their component building blocks. For example, proteins are degraded to amino acids, polysaccharides to monosaccharides, and fats (triacylglycerols) to free fatty acids and glycerol.

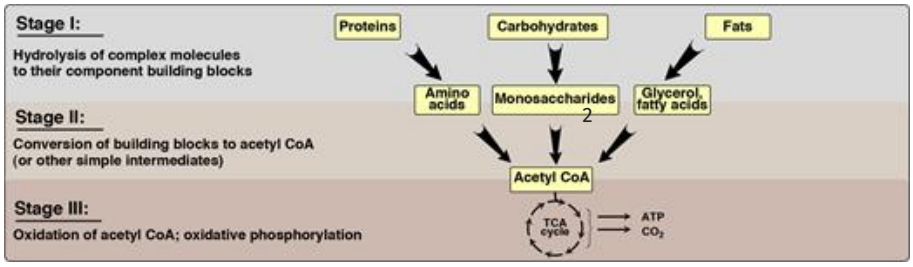
Conv

In the second stage, these diverse building blocks are further degraded to acetyl coenzyme A (CoA) and a few other simple molecules. Some energy is captured as ATP, but the amount is small compared with the energy produced during the third stage of catabolism.

Oxidation of acetyl CoA

The tricarboxylic acid (TCA) cycle (see Chapter 9) is the final common pathway in the oxidation of fuel molecules that produce acetyl CoA. Oxidation of acetyl CoA generates large amounts of ATP via oxidative phosphorylation as electrons flow from NADH and flavin adenine dinucleotide ($FADH_2$) to oxygen ($[O_2]$, see Chapter 6).

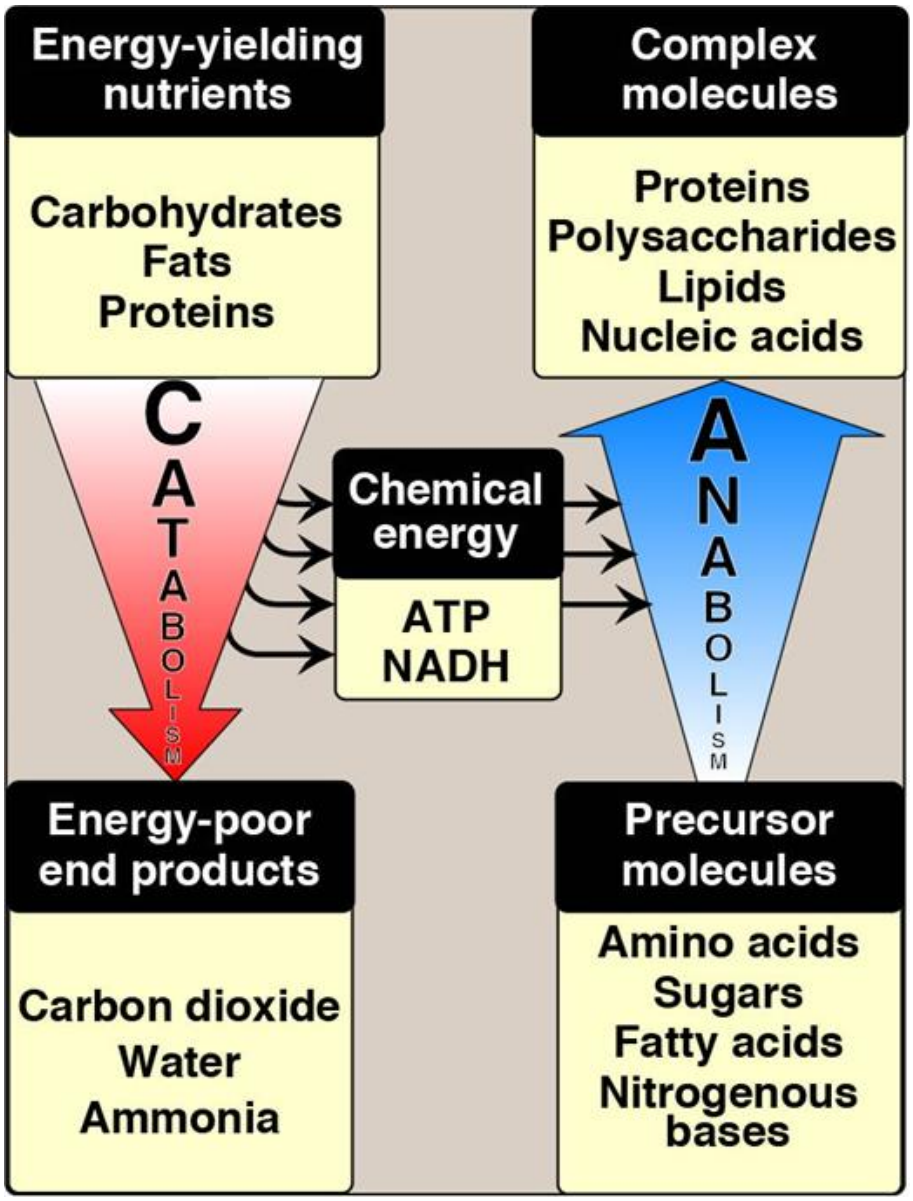
FIGURE 8.3



Anabolic pathways

In contrast to catabolism, anabolism is a divergent process in which a few biosynthetic precursors (such as amino acids) form a wide variety of polymeric, or complex, products (such as proteins [Fig. 8.4]). Anabolic reactions require energy (are endergonic), which is generally provided by the hydrolysis of ATP to adenosine diphosphate (ADP) and inorganic phosphate (P_i). (Note: Catabolic reactions generate energy [are exergonic].) Anabolic reactions often involve chemical reductions in which the reducing power is most frequently provided by the electron donor NADPH (phosphorylated NADH, see Chapter 13).

FIGURE 8.4

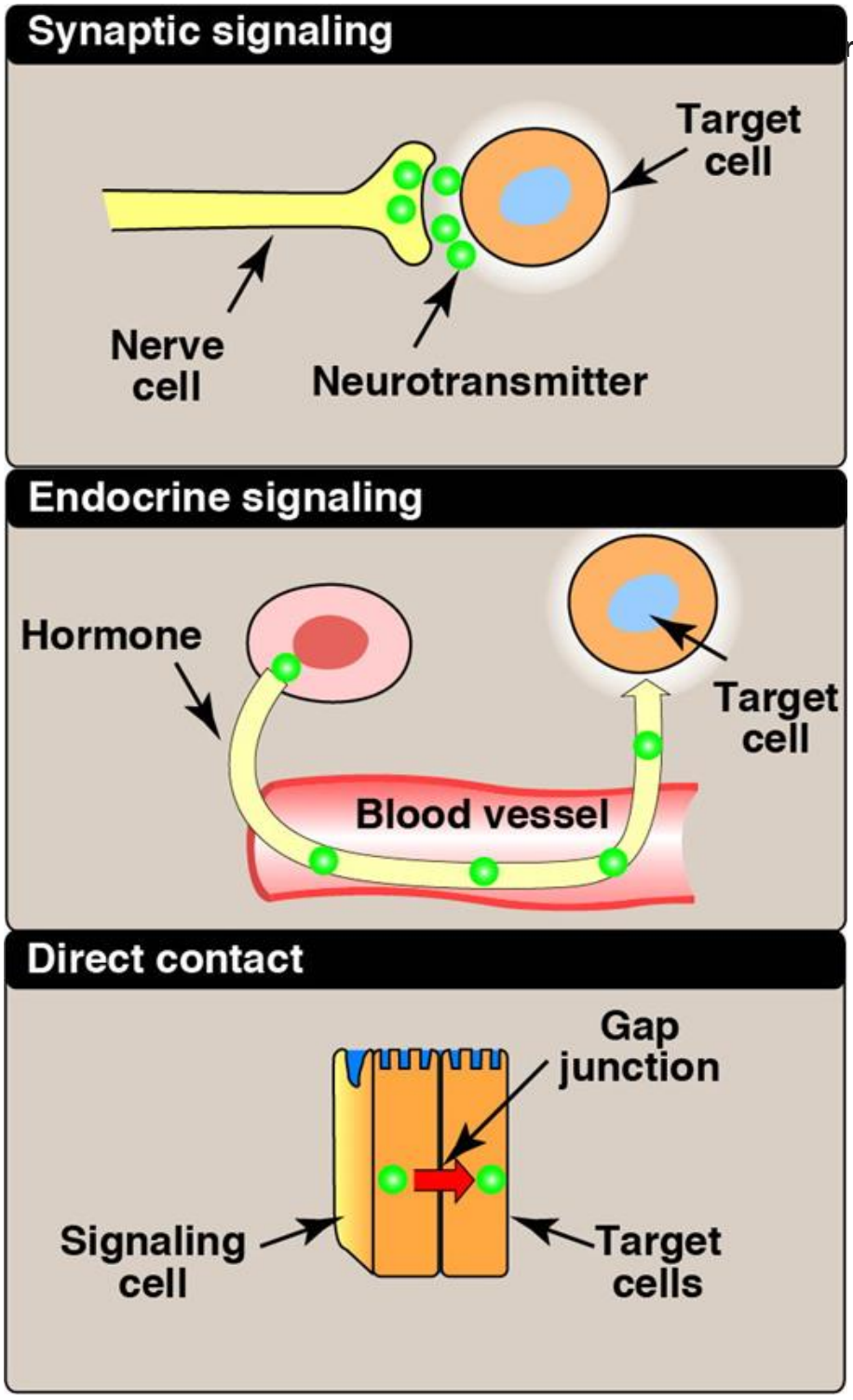


Metabolism Regulation

Listen

The pathways of metabolism must be coordinated so that the production of energy or the synthesis of end products meets the needs of the cell. Furthermore, individual cells function as part of a community of interacting tissues, not in isolation. Thus, a sophisticated communication system has evolved to coordinate the functions of the body. Regulatory signals that inform an individual cell of the metabolic state of the body as a whole include hormones, neurotransmitters, and the availability of nutrients. These, in turn, influence signals generated within the cell (Fig. 8.5).

FIGURE 8.5



ry signals between cells.

Intracellular communication

The rate of a metabolic pathway can respond to regulatory signals that arise from within the cell. For example, the rate may be influenced by the availability of substrates, product inhibition, or alterations in the levels of allosteric activators or inhibitors. These intracellular signals typically elicit rapid responses and are important for th

Inter

The ability to respond to intercellular signals is essential for the development and survival of organisms. Signaling between cells provides for long-range integration of metabolism and usually results in a response, such as a change in gene expression that is slower than is seen with intracellular signals. Communication between cells can be mediated, for example, by surface-to-surface contact and, in some tissues, by formation of gap junctions, allowing direct communication between the cytoplasms of adjacent cells. However, for energy metabolism, the most important route of communication is chemical signaling between cells by blood-borne hormones or by neurotransmitters.

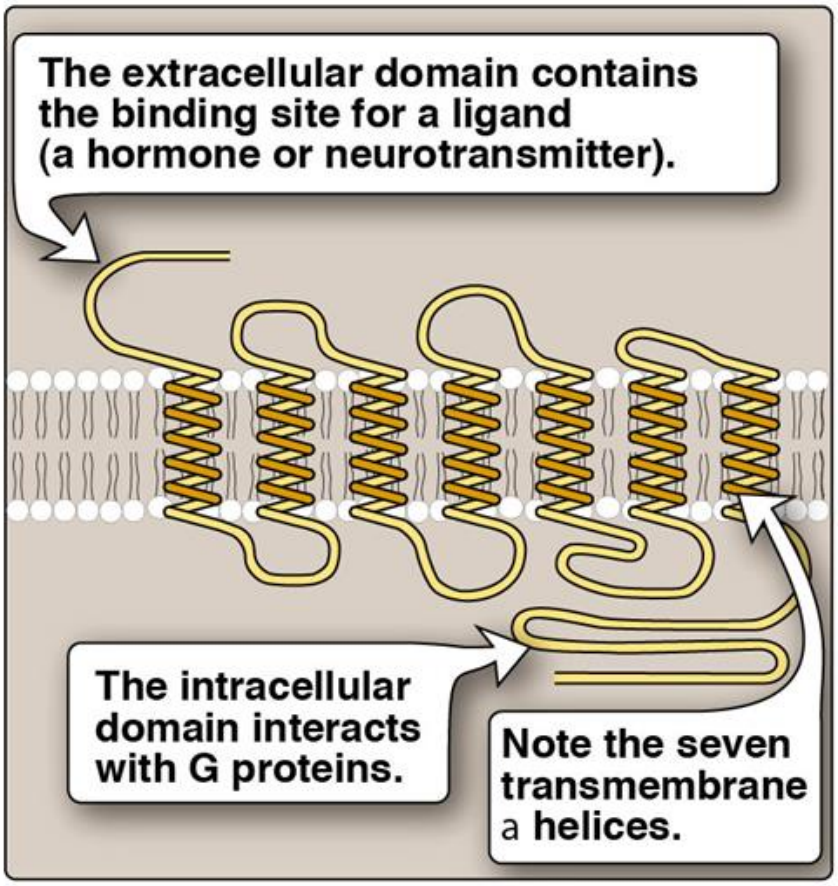
G protein–linked receptors and second messenger systems

Hormones and neurotransmitters can be thought of as signals and their receptors as signal detectors. Receptors are proteins often found embedded in the plasma membranes of their target cells. They respond to a ligand bound to them by initiating a series of reactions that ultimately result in specific intracellular responses. Many receptors that regulate metabolism are linked to intracellular GTP-binding proteins called G proteins and are known as G protein–coupled receptors (GPCRs). This type of receptor regulates production of molecules referred to as second messengers, which are so named because they intervene between the original extracellular messenger (the neurotransmitter or hormone) and the ultimate intracellular effect. Second messengers are part of the cascade of events that converts (transduces) ligand binding into a response.

Two of the most widely recognized second messenger systems regulated by G proteins are the phospholipase C system that involves calcium and phosphatidylinositol system and the adenylyl cyclase (adenylate cyclase) system, which is particularly important in regulating the pathways of intermediary metabolism. Both systems are initiated by the binding of hormone ligands, such as epinephrine or glucagon, to specific GPCR embedded with the plasma membrane of the target cell that will respond to the hormone.

GPCRs are characterized by an extracellular ligand-binding domain, seven transmembrane α helices, and an intracellular domain that interacts with heterotrimeric G proteins composed of α, β, and γ subunits (Fig. 8.6). (Note: Insulin, another key regulator of metabolism, does not signal via GPCRs but instead acts via a receptor with tyrosine kinase activity [see Chapter 23].[a])

FIGURE 8.6



membrane.

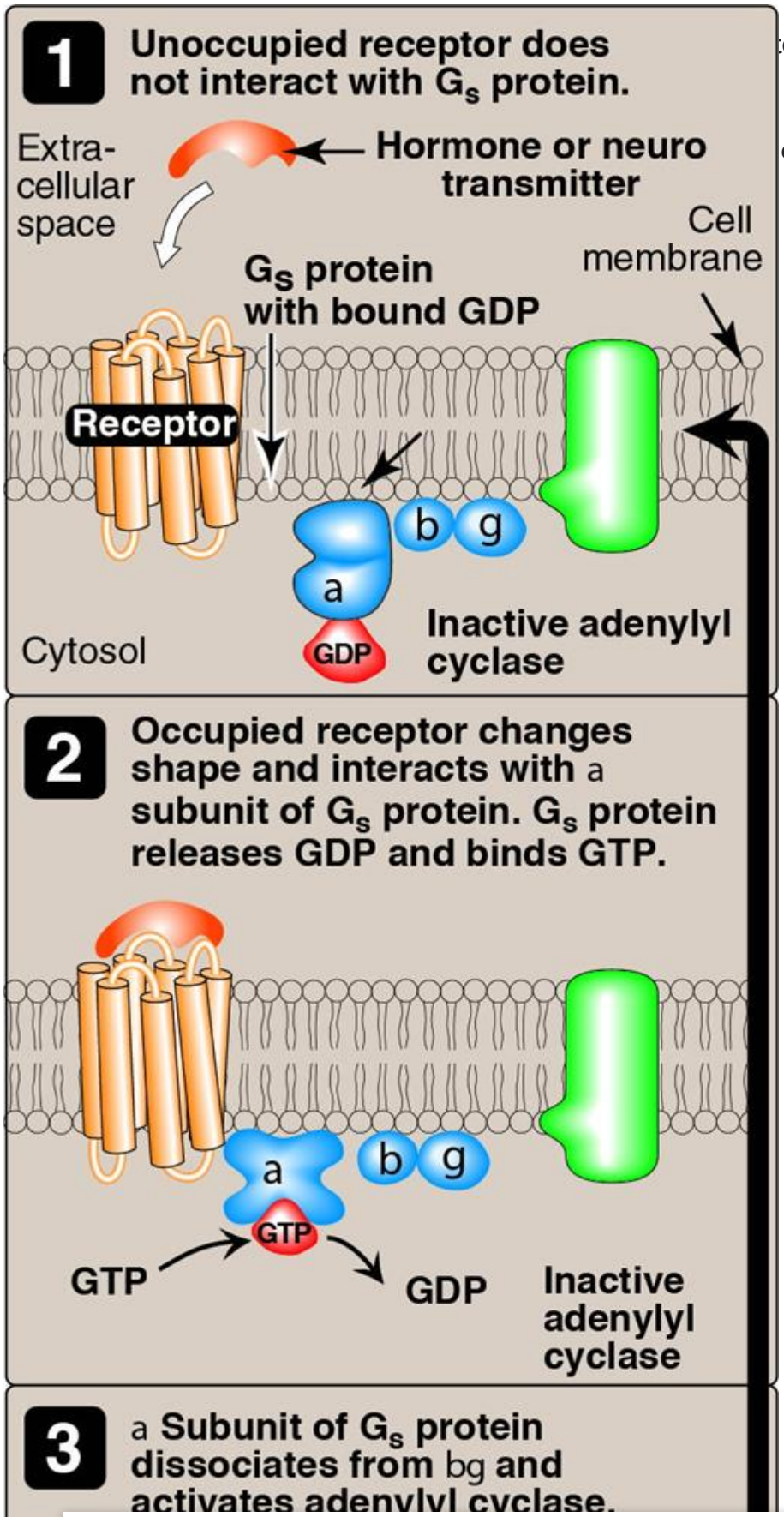
Adenylyl cyclase

Binding of the hormone ligand by some GPCRs, including the β- and α_2-adrenergic receptors, triggers either an increase or a decrease in the activity of adenylyl cyclase. This is a membrane-bound enzyme that converts ATP to 3′,5′-adenosine monophosphate (cyclic AMP, or cAMP) when active.

Guanosine triphosphate–dependent regulatory proteins or G proteins

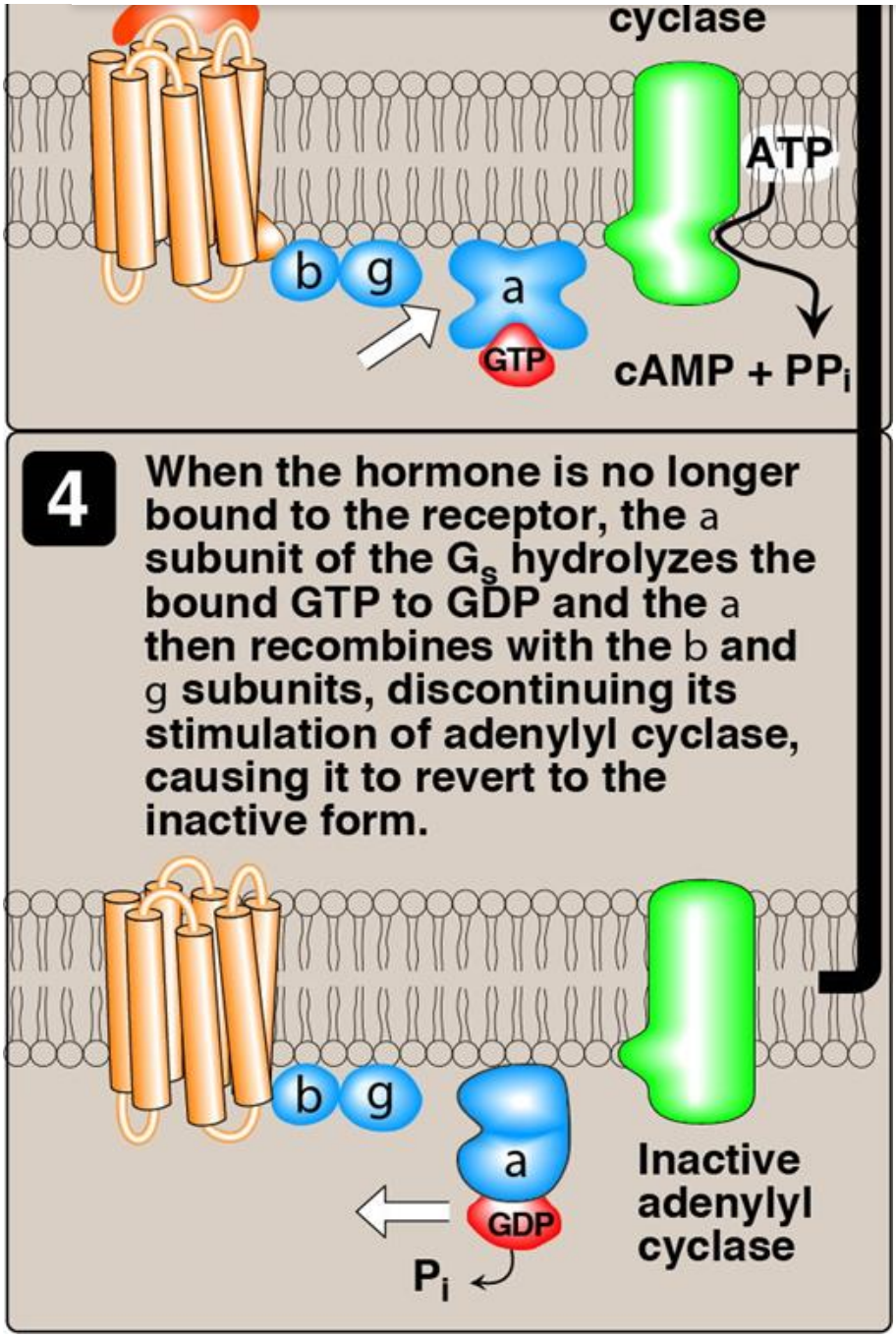
The effect of the activated, occupied GPCR on second messenger formation is mediated by specialized heterotrimeric G proteins (α, β, and γ subunits) found on the inner face of the plasma membrane. G proteins are named because their α subunit binds guanosine triphosphate (GTP) when activated. In the inactive form of a G protein, the α subunit is bound to GDP (Fig. 8.7). Ligand binding causes a conformational change in the receptor, triggering replacement of this GDP with GTP. The GTP-bound form of the α subunit dissociates from the βγ subunits and moves to the membrane-bound adenylyl cyclase enzyme, affecting its enzyme activity. Many molecules of active Gα protein are formed by one activated receptor. (Note: The ability of a hormone or neurotransmitter to stimulate or inhibit adenylyl cyclase depends on the type of Gα protein that is linked to the receptor. One type, designated G_s, stimulates adenylyl cyclase [see Fig. 8.7] whereas Gi inhibits adenylyl cyclase [not shown].)

FIGURE 8.7



tors triggers an increase (or, less

e monophosphate.



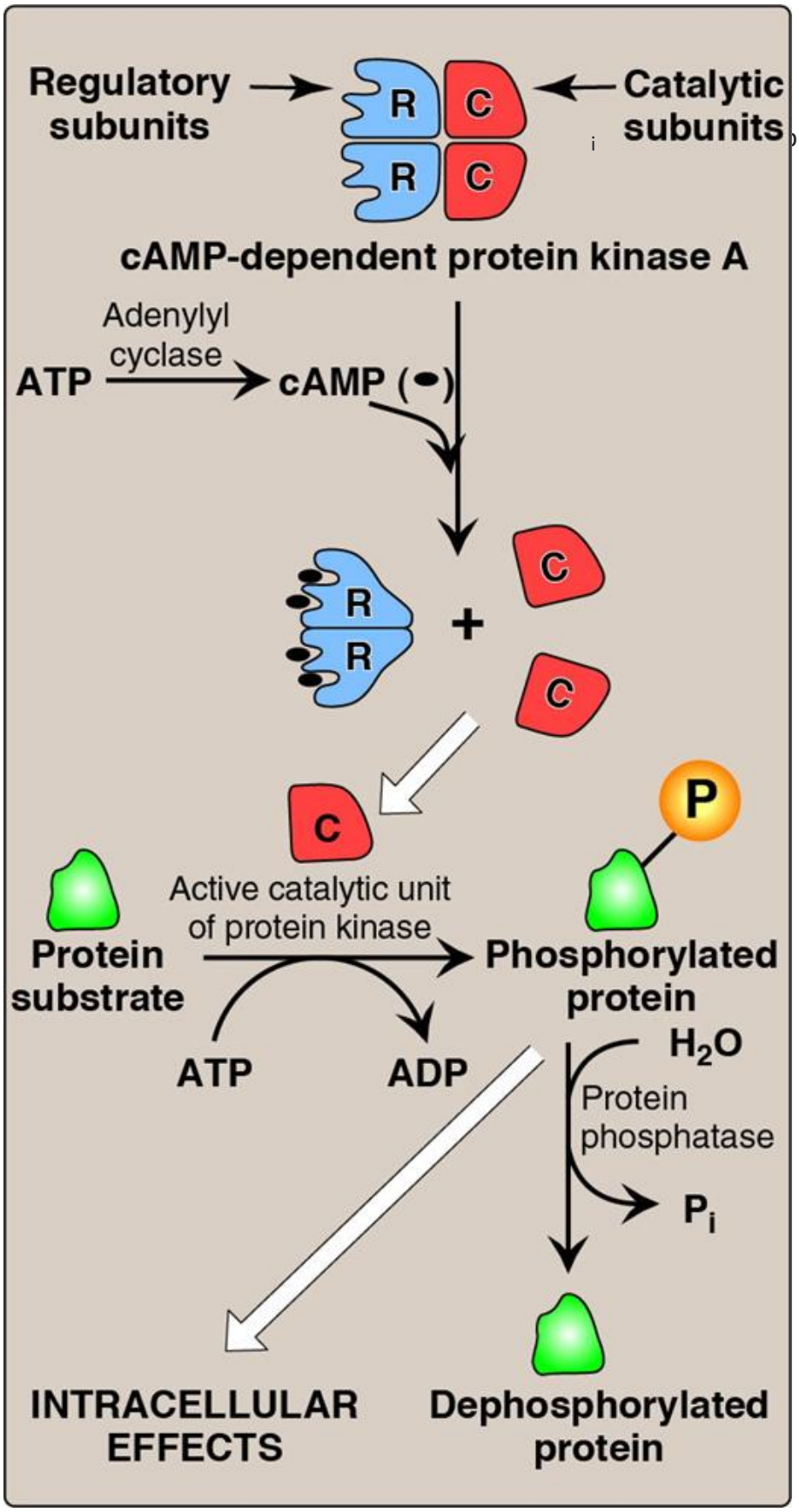
Activated adenylyl cyclase converts adenosine triphosophate (ATP) to the second messenger cAMP or cyclic adenosine monophosphate. cAMP then activates the serine/threonine protein kinase known as protein kinase A (PKA), described below. The actions of the Gα–GTP complex are short-lived because Gα has an inherent GTPase activity, resulting in the rapid hydrolysis of GTP to GDP. This causes inactivation of Gα, its dissociation from adenylyl cyclase, and its reassociation with the βγ dimer.

Toxins from *Vibrio cholerae* (cholera) and *Bordetella pertussis* (whooping cough) cause inappropriate activation of adenylyl cyclase through covalent modification (ADP-ribosylation) of different G proteins that interact with adenylyl cyclase. With cholera toxin, the GTPase activity of $G\alpha_s$ is inhibited in intestinal cells. With whooping cough, the pertussis toxin inactivates $G\alpha_i$ in respiratory tract cells

Prote

The next step in the cAMP second messenger system is the activation of a family of enzymes called cAMP-dependent protein kinases, including PKA, as shown in Figure 8.8. cAMP activates PKA by binding to its two regulatory subunits, causing the release of its two catalytically active subunits. Active PKA is a serine/threonine kinase because it functions to transfer phosphate from ATP to specific serine or threonine residues of its specific protein substrates. The phosphorylated proteins may act directly on the cell's ion channels or, if enzymes, may become activated or inhibited. (Note: Not all types of protein kinases are cAMP dependent, e.g., protein kinase C, activated in response to phospholipase C signaling, is calcium dependent.)

FIGURE 8.8



hate.

Prote

The phosphate groups added to proteins by protein kinases are removed by phosphoprotein phosphatases, enzymes that hydrolytically cleave phosphate esters (see Fig. 8.8). Actions of phosphatases ensure that changes in protein activity induced by phosphorylation are not permanent.

cAMP hydrolysis

cAMP is rapidly hydrolyzed to 5′-AMP by cAMP phosphodiesterase that cleaves the cyclic 3′,5′-phosphodiester bond. 5′-AMP is not an intracellular signaling molecule. Therefore, the effects of neurotransmitter- or hormone-mediated increases of cAMP are rapidly terminated if the extracellular signal is removed. (Note: cAMP phosphodiesterase is inhibited by caffeine, a methylxanthine derivative.)

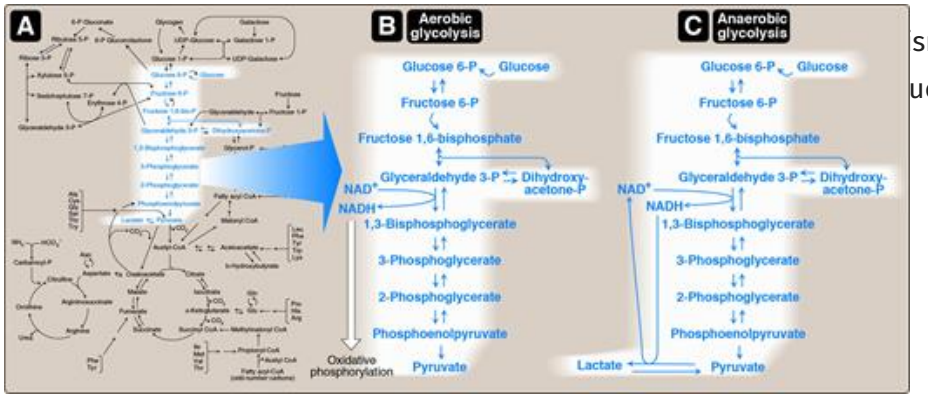
[a]For more information on GPCR signaling and second messengers, see *LIR Cell and Molecular Biology*, 2nd ed.

Glycolysis Overview

Listen

The glycolytic pathway is used by all tissues for the oxidation of glucose to provide energy (as ATP) and intermediates for other metabolic pathways. Glycolysis is at the hub of carbohydrate metabolism because virtually all sugars, whether arising from the diet or from catabolic reactions in the body, can ultimately be converted to glucose (Fig. 8.9A). Pyruvate is the end product of glycolysis in cells with mitochondria and an adequate supply of O_2. This series of 10 reactions is called aerobic glycolysis because O_2 is required to reoxidize the NADH formed during the oxidation of glyceraldehyde 3-phosphate (Fig. 8.9B). Aerobic glycolysis sets the stage for the oxidative decarboxylation of pyruvate to acetyl CoA, a major fuel of the TCA cycle. Alternatively, pyruvate is reduced to lactate as NADH is oxidized to NAD^+ (Fig. 8.9C). This conversion of glucose to lactate is called anaerobic glycolysis because it can occur without the participation of O_2. Anaerobic glycolysis allows the production of ATP in tissues that lack mitochondria (e.g., red blood cells [RBCs] and parts of the eye) or in cells deprived of sufficient O_2 (hypoxia).

FIGURE 8.9



sm. **B:** Reactions of aerobic glycolysis. ucleotide; P = phosphate.

Glucose Transport into Cells

Glucose cannot diffuse directly into cells but enters by one of two transport systems: a sodium (Na^+)- and ATP-independent transport system or a Na^+- and ATP-dependent cotransport system.

Sodium- and ATP-independent transport system

This passive system is mediated by a family of 14 glucose transporter (GLUT) isoforms found in cell membranes. They are designated GLUT-1 to GLUT-14. These monomeric protein transporters exist in the membrane in two conformational states (Fig. 8.10). Extracellular glucose binds to the transporter, which then alters its conformation, transporting glucose across the cell membrane via facilitated diffusion. Because GLUTs transport one molecule at a time, they are uniporters.[b]

Tissue specificity

GLUT display a tissue-specific pattern of expression (See Table 8.1 for examples of some GLUTs). For example, GLUT-1 is abundant in most tissues, whereas GLUT-4 is abundant in muscle and adipose tissue, and GLUT-5 transports fructose. (Note: The number of GLUT-4 transporters active in these tissues is increased by insulin [see p. 345 for a discussion of insulin and glucose transport].) GLUT-2 is abundant in the liver, kidneys, and pancreatic β cells. The other GLUT isoforms also have tissue-specific distributions.

TABLE 8.1

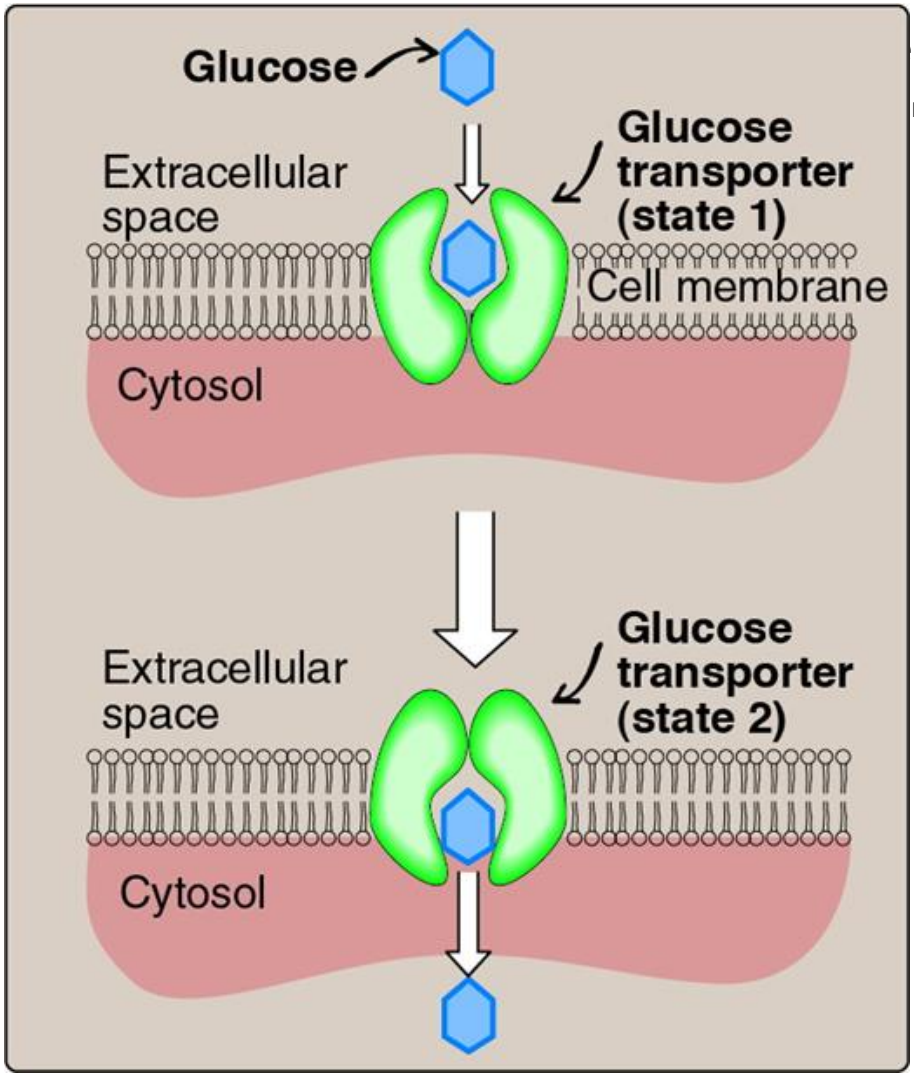
Tissue Distribution of Selected GLUTs

	Location	Function	K_m (mM)
GLUT-1	Most tissues	Basal glucose uptake	1
GLUT-2	Liver, kidneys, pancreas	Removes excess glucose from blood	15–20
GLUT-3	Most tissues	Basal glucose uptake	1
GLUT-4	Muscle and fat	Removes excess glucose from blood	5
GLUT-5	Small intestine, testes	Transport of fructose	10

Specialized functions

In facilitated diffusion, transporter-mediated glucose movement is down a concentration gradient (i.e., from a high concentration to a lower one, therefore requiring no energy). For example, GLUT-1, GLUT-3, and GLUT-4 are primarily involved in glucose uptake from the blood. In contrast, GLUT-2, in the liver and kidneys, can either transport glucose into these cells when blood glucose levels are high or transport glucose from these cells when blood glucose levels are low (e.g., during fasting). GLUT-5 is unusual in that it is the primary transporter for fru

FIGURE 8.10



through a cell membrane.

membrane α helices.)

Sodium- and ATP-dependent cotransport of glucose

This type of glucose cotransport with sodium occurs in the epithelial cells of the intestine, the renal tubules, and the choroid plexus. This is an energy-requiring process that transports glucose against (up) its concentration gradient, from low extracellular concentrations to higher intracellular concentrations while Na^+ is transported down its electrochemical gradient. There is a much higher extracellular than intracellular concentration of Na^+, which is the result of the Na^+–K^{++} ATPase. The Na^+ concentration gradient powers the transport of glucose against its concentration gradient; ATP hydrolysis is an indirect energy source because it is necessary to establish the Na^+ gradient. (see also Fig. 7.10). Because this secondary active transport of glucose requires the concurrent uptake (symport) of Na^+, the transporter is a sodium-dependent glucose cotransporter (SGLT). (Note: The choroid plexus, part of the blood–brain barrier, also contains GLUT-1.)[c]

Sodium-dependent glucose cotransporter protein 2 (SGLT2) functions in the kidneys, and is the major transporter for glucose reabsorption back into the blood. Gliflozins are SGLT2 inhibitors, which reduce reabsorption of glucose in the kidney, and therefore lowe

[b]For more information on glucose transport, see *LIR Cell and Molecular Biology*, 2nd Ed., Chapter 15.

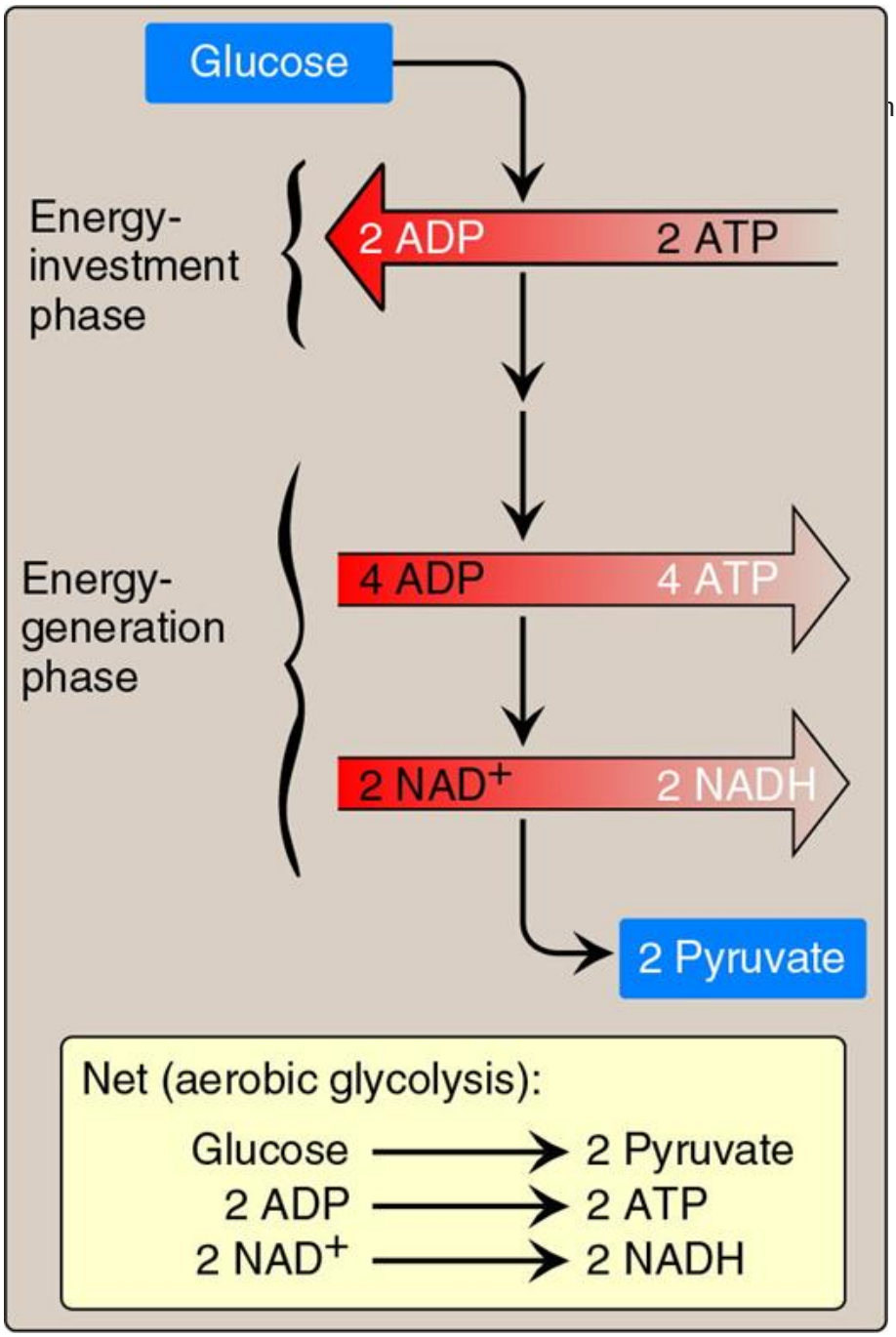
[c]For further information, see *LIR Cell and Molecular Biology*, 2nd Editions, Chapters 14 and 15.

Glycolysis Reactions

Listen

The conversion of glucose to pyruvate occurs in two stages (Fig. 8.11). The first five reactions of glycolysis correspond to an energy-investment phase in which the phosphorylated forms of intermediates are synthesized at the expense of ATP. The subsequent reactions of glycolysis constitute an energy-generation phase in which a net of two molecules of ATP are formed by substrate-level phosphorylation per glucose molecule metabolized.

FIGURE 8.11



hate.

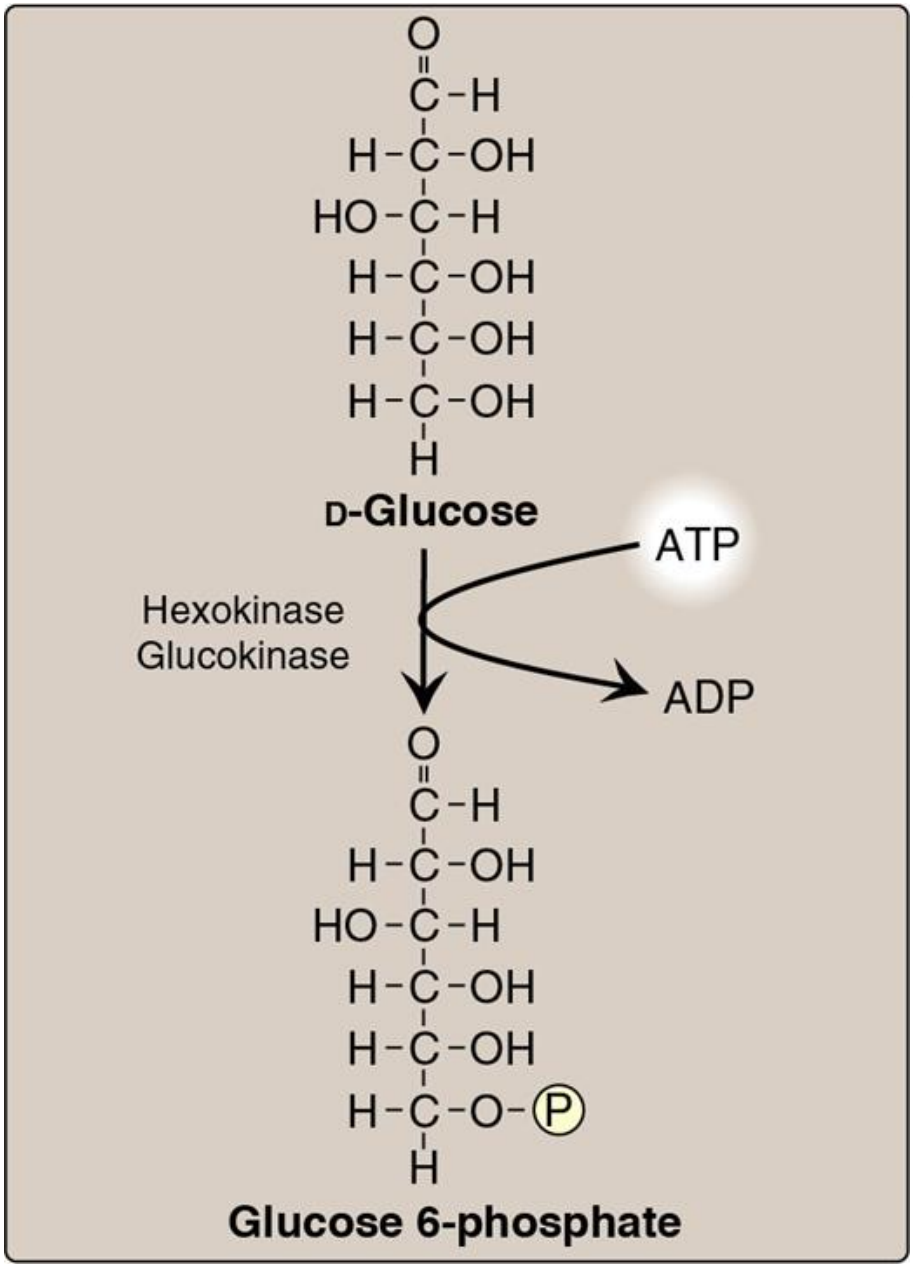
Glucose phosphorylation

Phosphorylated sugar molecules do not readily penetrate cell membranes because there are no specific transmembrane carriers for these compounds and because they are too polar to diffuse through the lipid core of membranes. Therefore, the irreversible phosphorylation of glucose (Fig. 8.12) effectively traps the sugar as cytosolic glucose 6-phosphate and commits it to further metabolism in the cell. Mammals have four isozymes (I–IV) of the enzyme hexokinase that catalyze the phosphorylation of glucose to glucose 6-phosphate.

Hexo

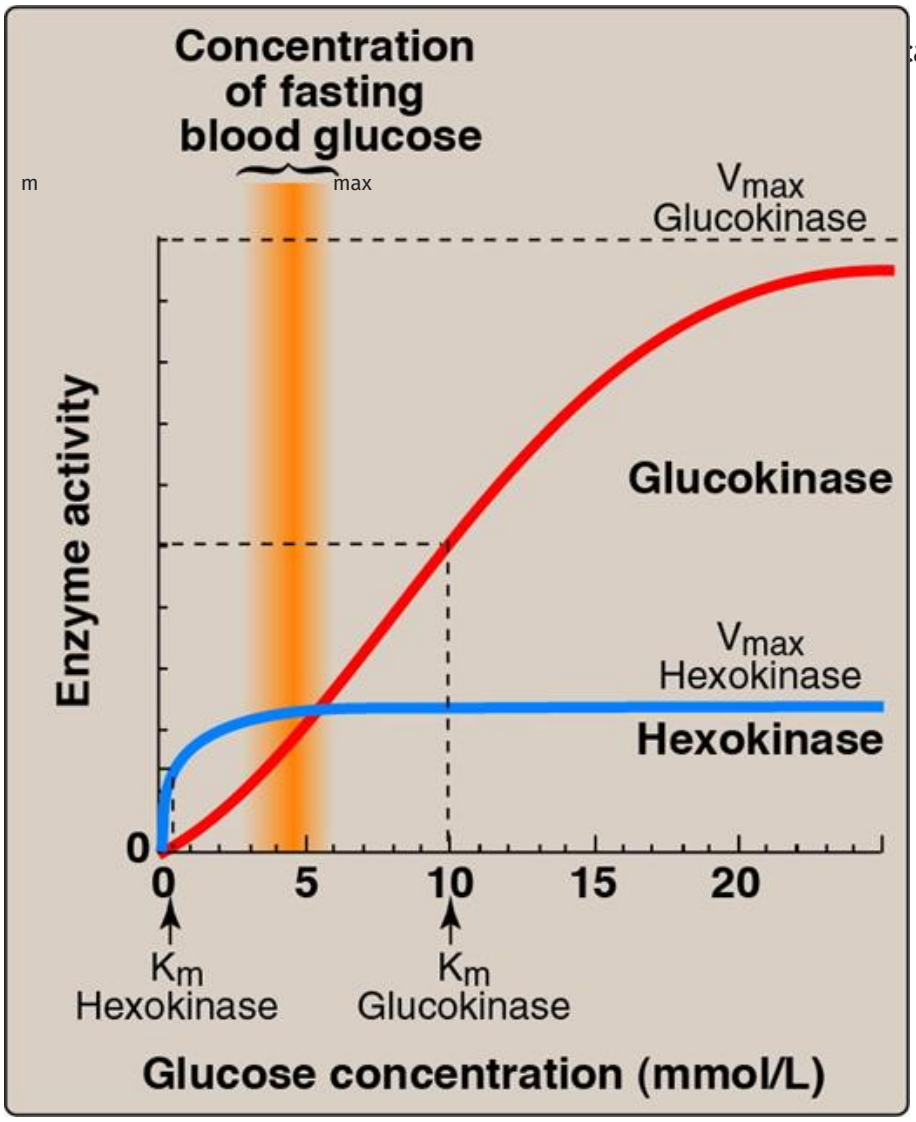
In most tissues, glucose phosphorylation is catalyzed by one of these isozymes of hexokinase, which is one of three regulatory enzymes of glycolysis (along with phosphofructokinase [PFK] and pyruvate kinase [PK]). They are inhibited by the reaction product glucose 6-phosphate, which accumulates when further metabolism of this hexose phosphate is reduced. Hexokinases I–III have a low Michaelis constant (K_m) and, therefore, a high affinity (see p. 63) for glucose. This permits the efficient phosphorylation and subsequent metabolism of glucose even when tissue concentrations of glucose are low (Fig. 8.13). However, because these isozymes have a low maximal velocity ([V_{max}], see p. 61) for glucose, they do not sequester (trap) cellular phosphate in the form of phosphorylated glucose or phosphorylate more glucose than the cell can use. (Note: These isozymes have broad substrate specificity and are able to phosphorylate several hexoses in addition to glucose.)

FIGURE 8.12



ically magnesium.) ADP = adenosine

FIGURE 8.13



atalyzed by hexokinase and

Hexokinase IV

In liver parenchymal cells and pancreatic β cells, glucokinase (the hexokinase IV isozyme) is the predominant enzyme responsible for glucose phosphorylation. In β cells, glucokinase functions as a glucose sensor, determining the threshold for insulin secretion (see p. 343). (Note: Hexokinase IV also serves as a glucose sensor in hypothalamic neurons, playing a key role in the adrenergic response to hypoglycemia [see p. 350].) In the liver, the enzyme facilitates glucose phosphorylation during hyperglycemia. Despite the popular but misleading name glucokinase, the sugar specificity of the enzyme is similar to that of other hexokinase isoenzymes.

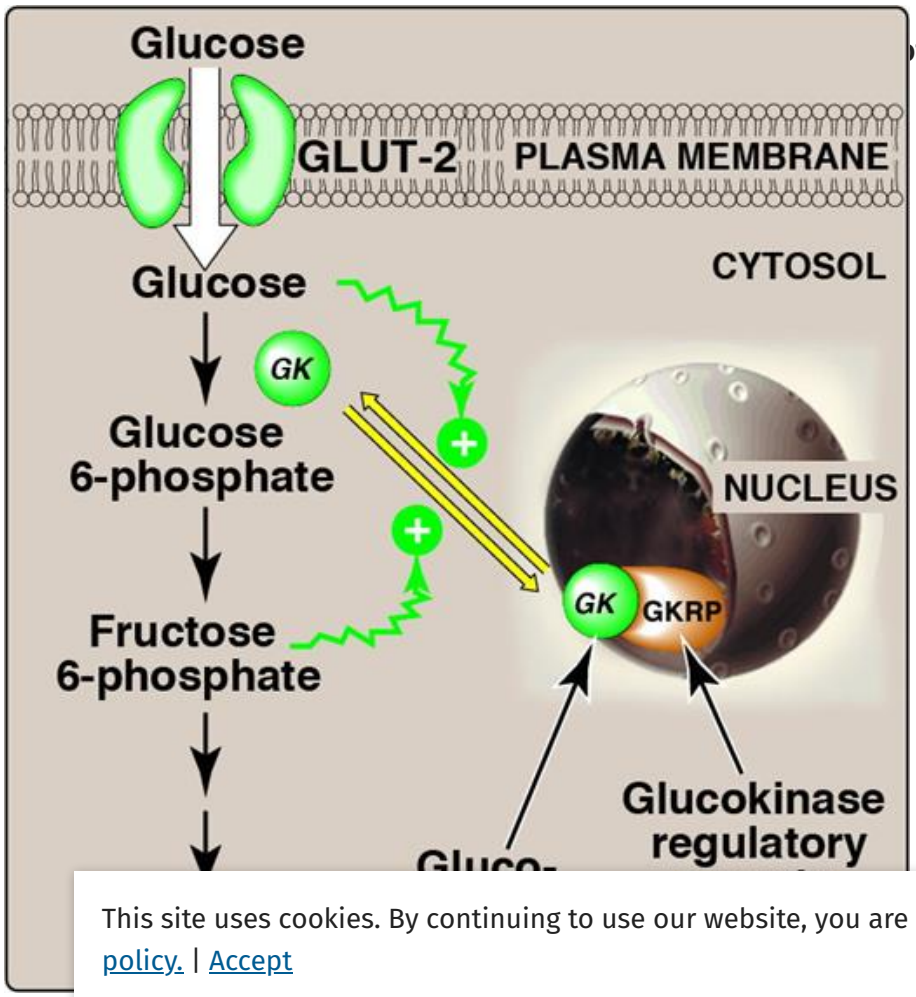
Kinetics

Glucokinase differs from hexokinases I–III in several important properties. For example, it has a much higher K_m, requiring a higher glucose concentration for half-saturation (see Fig. 8.13). Thus, glucokinase functions only when the intracellular concentration of glucose in the hepatocyte is elevated such as during the brief period following consumption of a carbohydrate-rich meal, when high levels of glucose are delivered to the liver via the portal vein. Glucokinase has a high V_{max}, allowing the liver to effectively remove the flood of glucose delivered by the portal blood. This prevents large amounts of glucose from entering the systemic circulation following such a meal, thereby minimizing hyperglycemia during the absorptive period. (Note: GLUT-2 ensures that blood glucose equilibrates rapidly across the hepatocyte membrane.)

Regulation

Glucokinase activity is not directly inhibited by glucose 6-phosphate as are the other hexokinases. Instead, it is indirectly inhibited by fructose 6-phosphate (which is in equilibrium with glucose 6-phosphate, a product of glucokinase) and is indirectly stimulated by glucose (a substrate of glucokinase). Regulation is achieved by reversible binding to the hepatic protein glucokinase regulatory protein (GKRP). In the presence of fructose 6-phosphate, glucokinase binds tightly to GKRP and is translocated to the nucleus, thereby rendering the enzyme inactive (Fig. 8.14). When glucose levels in the blood (and also in the hepatocyte, as a result of GLUT-2) increase, glucokinase is released from GKRP, and the enzyme reenters the cytosol where it phosphorylates glucose to glucose 6-phosphate. (Note: GKRP is a competitive inhibitor of glucose use by glucokinase.)

FIGURE 8.14



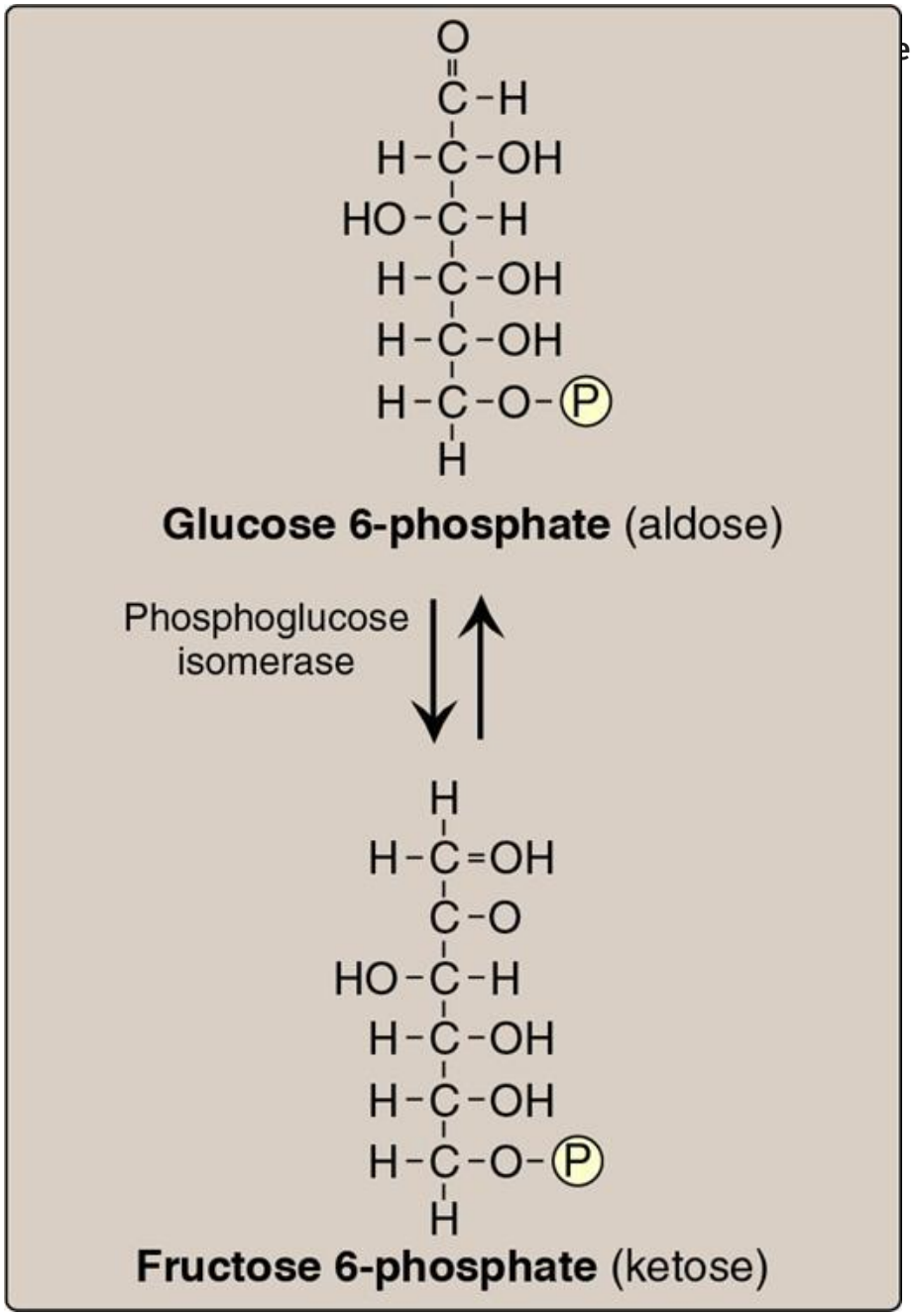
tein.

Glucokinase functions as a glucose sensor in blood glucose homeostasis. Inactivating mutations of glucokinase are the cause of a rare form of diabetes, maturity-onset diabetes of the young type 2 (MODY 2) that is characterized by impaired insulin secretion and hyperglycemia.

Glucose 6-phosphate isomerization

The isomerization of glucose 6-phosphate to fructose 6-phosphate is catalyzed by phosphoglucose isomerase (Fig. 8.15). The reaction is readily reversible and is not a rate-limiting or regulated step.

FIGURE 8.15



e 6-phosphate.

Fruc

The irreversible phosphorylation reaction catalyzed by PFK-1 is the most important control point and the rate-limiting and committed step of glycolysis (Fig. 8.16). PFK-1 is controlled by the available concentrations of the substrates ATP and fructose 6-phosphate as well as by other regulatory molecules.

Regulation by intracellular energy levels

PFK-1 is inhibited allosterically by elevated levels of ATP, which act as an energy-rich signal indicating an abundance of high-energy compounds. Elevated levels of citrate, an intermediate in the TCA cycle (see p. 122), also inhibit PFK-1. (Note: Inhibition by citrate favors the use of glucose for glycogen synthesis [see p. 138].) Conversely, PFK-1 is activated allosterically by high concentrations of AMP, which signal that the cell's energy stores are depleted.

Regulation by fructose 2,6-bisphosphate

Fructose 2,6-bisphosphate is the most potent activator of PFK-1 (see Fig. 8.16) and is able to activate the enzyme even when ATP levels are high. It is formed from fructose 6-phosphate by PFK-2. Unlike PFK-1, PFK-2 is a bifunctional protein that has both the kinase activity that produces fructose 2,6-bisphosphate and the phosphatase activity that dephosphorylates fructose 2,6-bisphosphate to fructose 6-phosphate. In the liver isozyme, phosphorylation of PFK-2 inactivates the kinase domain and activates the phosphatase domain (Fig. 8.17). The opposite is seen in the cardiac isozyme. Skeletal PFK-2 is not covalently regulated. (Note: Fructose 2,6-bisphosphate is an inhibitor of fructose 1,6-bisphosphatase, an enzyme of gluconeogenesis. The reciprocal actions of fructose 2,6-bisphosphate on glycolysis [activation] and gluconeogenesis [inhibition] ensure that both pathways are not fully active at the same time, preventing a futile cycle of glucose oxidation to pyruvate followed by glucose resynthesis from pyruvate.)

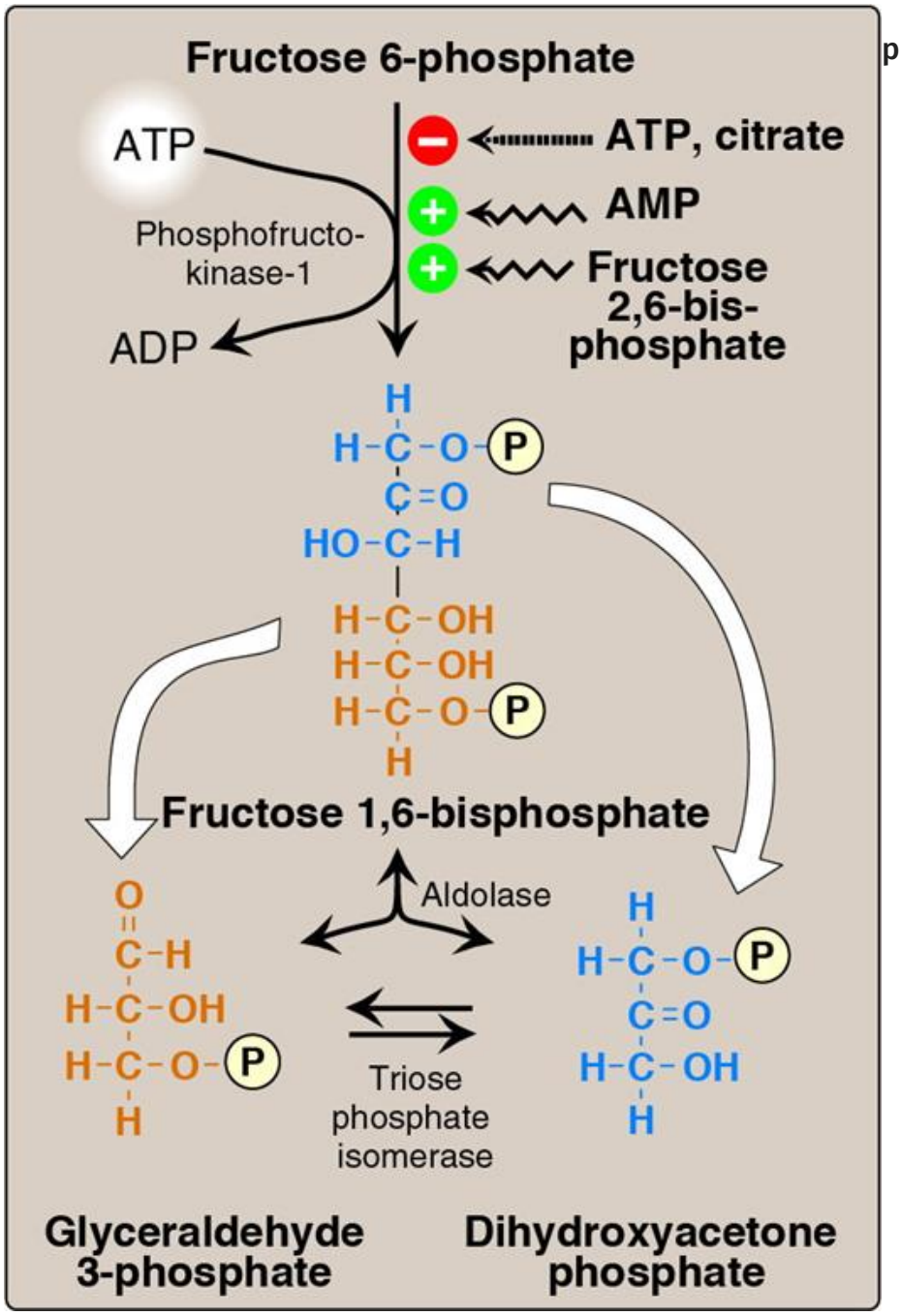
During the well-fed state

Decreased levels of glucagon and elevated levels of insulin (such as occur following a carbohydrate-rich meal) cause an increase in hepatic fructose 2,6-bisphosphate (PFK-2 is dephosphorylated) and, thus, in the rate of glycolysis (see Fig. 8.17). Therefore, fructose 2,6-bisphosphate acts as an intracellular signal of glucose abundance.

During fasting

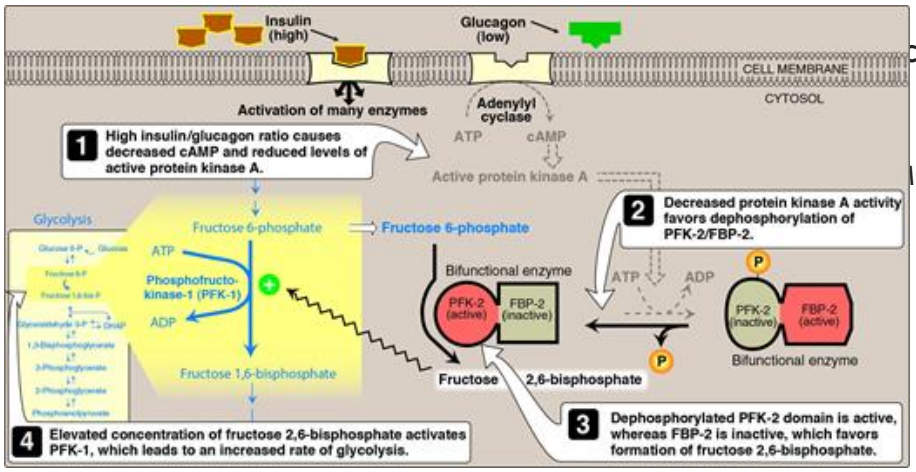
By contrast, the elevated levels of glucagon and low levels of insulin that occur during fasting (see p. 364) cause a decrease in hepatic fructose 2,6-bisphosphate (PFK-2 is phosphorylated). This results in inhibition of glycolysis and activation of gluconeogenesis.

FIGURE 8.16



phosphate to triose phosphates.

FIGURE 8.17



centration of fructose 2,6-

P and ADP = adenosine mono- and

Fructose 1,6-bisphosphate cleavage

Aldolase cleaves fructose 1,6-bisphosphate to dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (Fig. 8.16). The reaction is reversible and not regulated. (Note: Aldolase B, the hepatic isoform, also cleaves fructose 1-phosphate and functions in dietary fructose metabolism.)

Dihydroxyacetone phosphate isomerization

Triose phosphate isomerase interconverts DHAP and glyceraldehyde 3-phosphate (Fig. 8.16). DHAP must be isomerized to glyceraldehyde 3-phosphate for further metabolism by the glycolytic pathway. This isomerization results in the net production of two molecules of glyceraldehyde 3-phosphate from the cleavage products of fructose 1,6-bisphosphate. (Note: DHAP is utilized in triacylglycerol synthesis.)

Glyceraldehyde 3-phosphate oxidation

The conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate (1,3-BPG) by glyceraldehyde 3-phosphate dehydrogenase is the first oxidation–reduction reaction of glycolysis (Fig. 8.18). (Note: Because there is a limited amount of NAD^+ in the cell, the NADH formed by the dehydrogenase reaction must be oxidized for glycolysis to continue. Two major mechanisms for oxidizing NADH to NAD^+ are the reduction of pyruvate to lactate by lactate dehydrogenase [LDH] anaerobic, and the electron transport chain ([ETC] aerobic). Because NADH cannot cross the inner mitochondrial membrane, the ETC requires the malate–aspartate and glycerol 3-phosphate substrate shuttles to move NADH reducing equivalents into the mitochondrial matrix.)

1,3-Bisphosphoglycerate synthesis

The oxidation of the aldehyde group of glyceraldehyde 3-phosphate to a carboxyl group is coupled to the attachment of P_i to the carboxyl group. This phosphate group, linked to carbon 1 of the 1,3-BPG product by a high-energy bond, conserves much of the free energy produced by the oxidation of glyceraldehyde 3-phosphate. This high-energy phosphate drives ATP synthesis in the next reaction of glycolysis.

CLINICAL APPLICATION 8.1

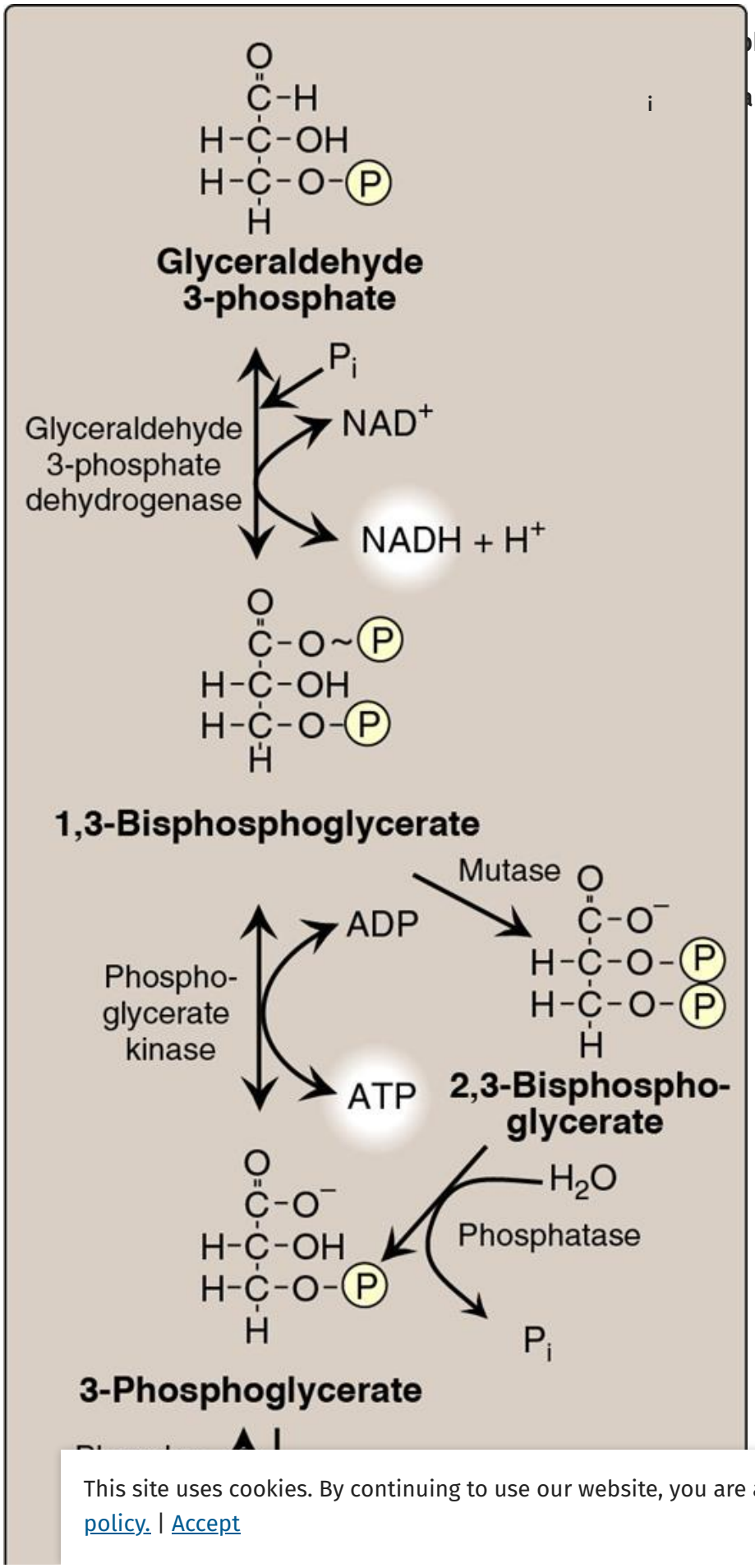
Arsenic Poisoning

The toxicity of arsenic is due primarily to the inhibition by trivalent arsenic (arsenite) of enzymes such as the pyruvate dehydrogenase complex (PDHC), which require lipoic acid as a coenzyme (see p. 121). However, pentavalent arsenic (arsenate) can prevent net ATP and NADH production by glycolysis without inhibiting the pathway itself. It does so by competing with P_i as a substrate for glyceraldehyde 3-phosphate dehydrogenase, forming a complex that spontaneously hydrolyzes to form 3-phosphoglycerate (see Fig. 8.18). By bypassing the synthesis of and phosphate transfer from 1,3-BPG, the cell is deprived of energy usually obtained from the glycolytic pathway. (Note: Arsenate also competes with P_i binding to the F_1 domain of ATP synthase resulting in formation of ADP-arsenate that is rapidly hydrolyzed.)

2,3-Bisphosphoglycerate synthesis in RBC

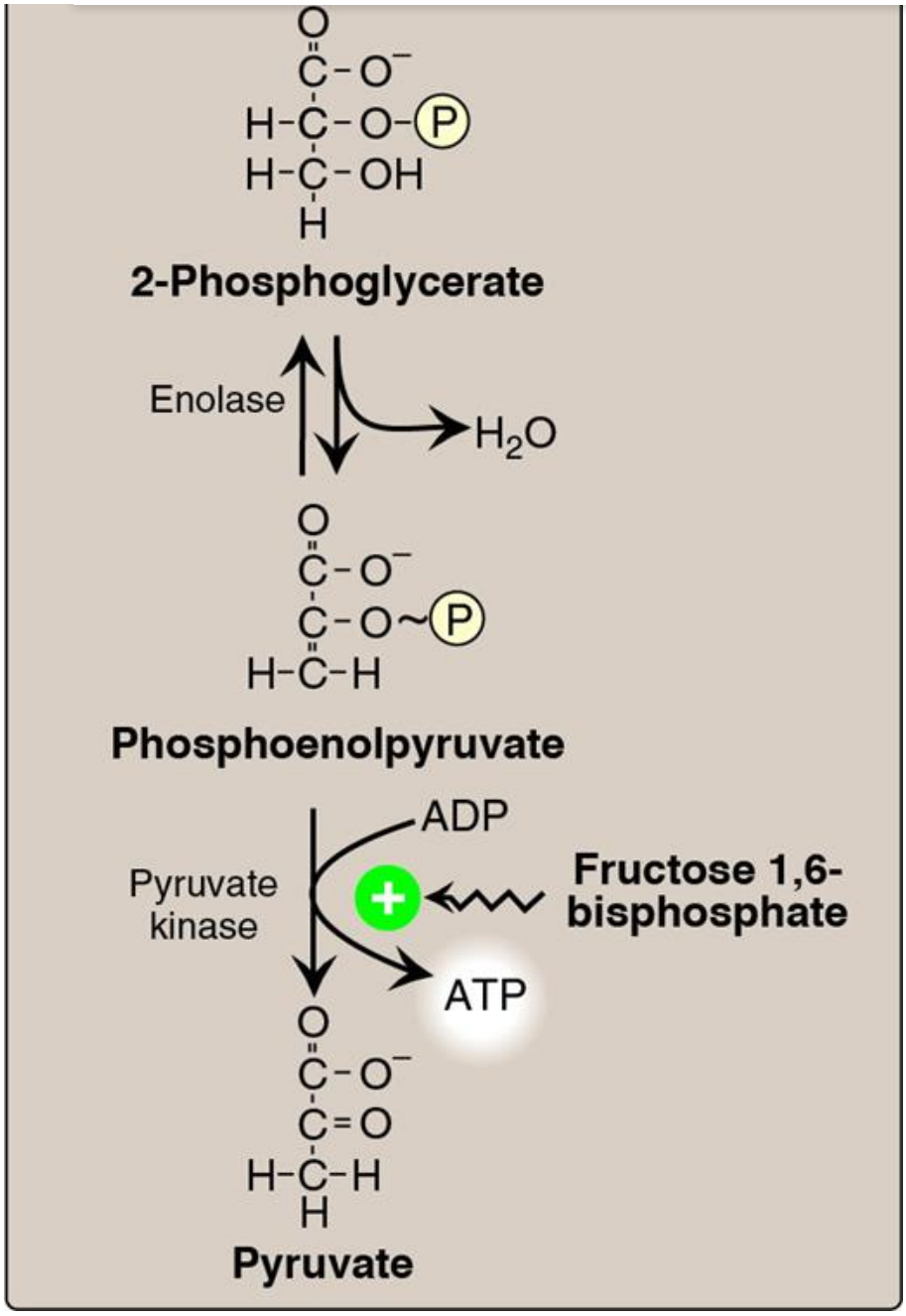
Some of the 1,3-BPG is converted to 2,3-BPG by the action of bisphosphoglycerate mutase (Fig. 8.18). 2,3-BPG, which is found in only trace amounts in most cells, is present at high concentration in RBC and serves to increase O_2 delivery. 2,3-BPG is hydrolyzed by a phosphatase to 3-phosphoglycerate, which is also an intermediate in glycolysis (Fig. 8.18). In the RBC, glycolysis is modified by inclusion of these shunt reactions.

FIGURE 8.18



hate to pyruvate. NAD(H) =

nic phosphate; ~ = high-energy



3-Phosphoglycerate synthesis and ATP production

When 1,3-BPG is converted to 3-phosphoglycerate, the high-energy phosphate group of 1,3-BPG is used to synthesize ATP from ADP (Fig. 8.18). This reaction is catalyzed by phosphoglycerate kinase, which, unlike most other kinases, is physiologically reversible. Because two molecules of 1,3-BPG are formed from each glucose molecule, this kinase reaction replaces the two ATP molecules consumed by the earlier formation of glucose 6-phosphate and fructose 1,6-bisphosphate. (Note: This reaction is an example of substrate-level phosphorylation, in which the energy needed for the production of a high-energy phosphate comes from a substrate rather than from the ETC [see J. below for other examples].)

Phos

The shift of the phosphate group from carbon 3 to carbon 2 of phosphoglycerate by phosphoglycerate mutase is freely reversible.

2-Phosphoglycerate dehydration

The dehydration of 2-phosphoglycerate by enolase redistributes the energy within the substrate, forming phosphoenolpyruvate (PEP), which contains a high-energy enol phosphate (Fig. 8.18). The reaction is reversible, despite the high-energy nature of the product. (Note: Fluoride inhibits enolase, and water fluoridation reduces lactate production by mouth bacteria, decreasing dental caries.)

Pyruvate synthesis and ATP production

The conversion of PEP to pyruvate, catalyzed by PK, is the third irreversible reaction of glycolysis. The high-energy enol phosphate in PEP is used to synthesize ATP from ADP and is another example of substrate-level phosphorylation (Fig. 8.18).

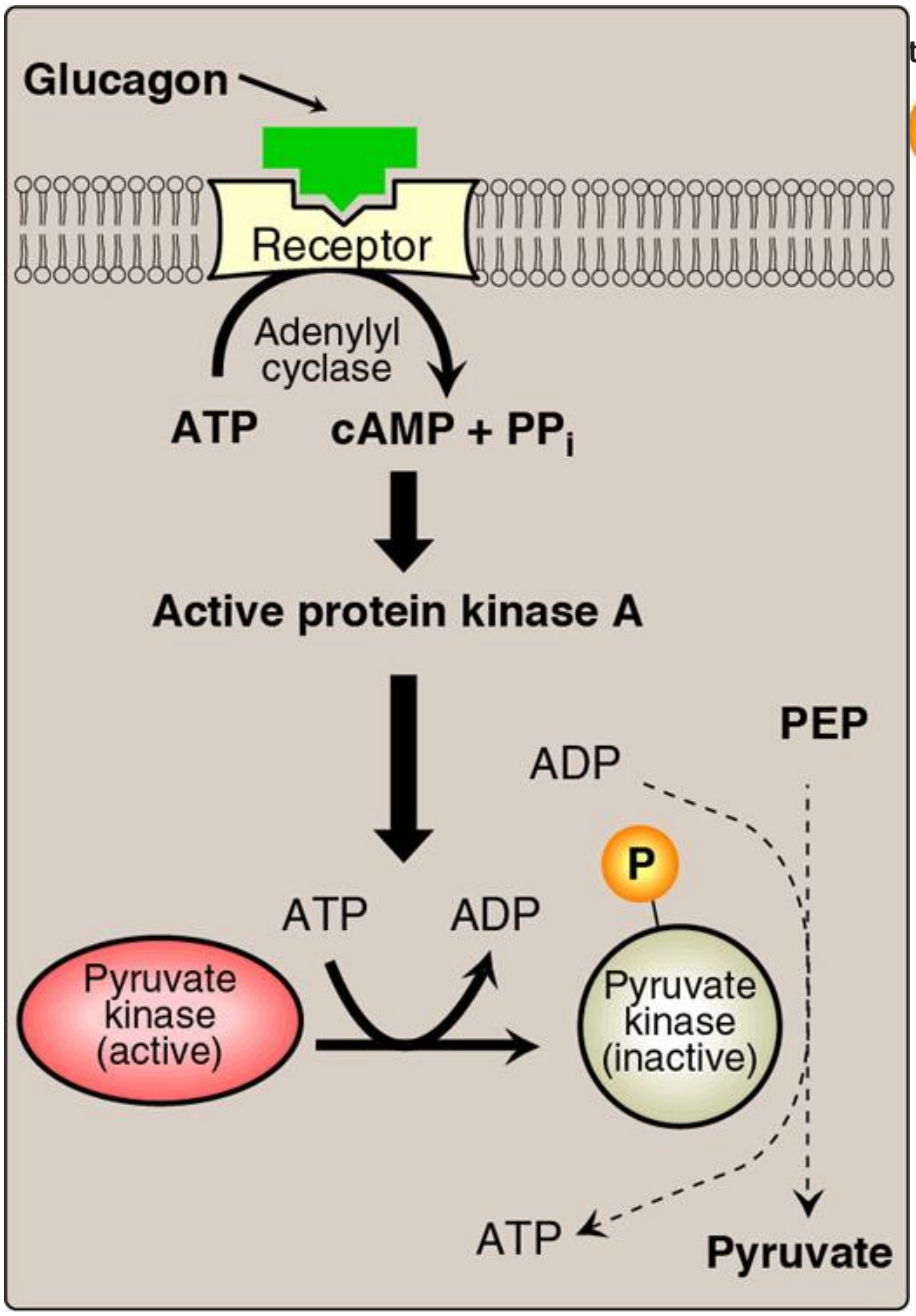
Feedforward regulation

PK is activated by fructose 1,6-bisphosphate, the product of the PFK-1 reaction. This feedforward (instead of the more usual feedback) regulation has the effect of linking the two kinase activities: increased PFK-1 activity results in elevated levels of fructose 1,6-bisphosphate, which activates PK. (Note: PK is inhibited by ATP.)

Covalent regulation in the liver

Phosphorylation by cAMP-dependent PKA leads to inactivation of the hepatic isozyme of PK (Fig. 8.19). When blood glucose levels are low, elevated glucagon increases the intracellular level of cAMP, which causes the phosphorylation and inactivation of PK in the liver only. Therefore, PEP is unable to continue in glycolysis and, instead, enters the gluconeogenesis pathway. This partly explains the observed inhibition of hepatic glycolysis and stimulation of gluconeogenesis by glucagon. Dephosphorylation of PK by a phosphatase results in reactivation of the enzyme.

FIGURE 8.19



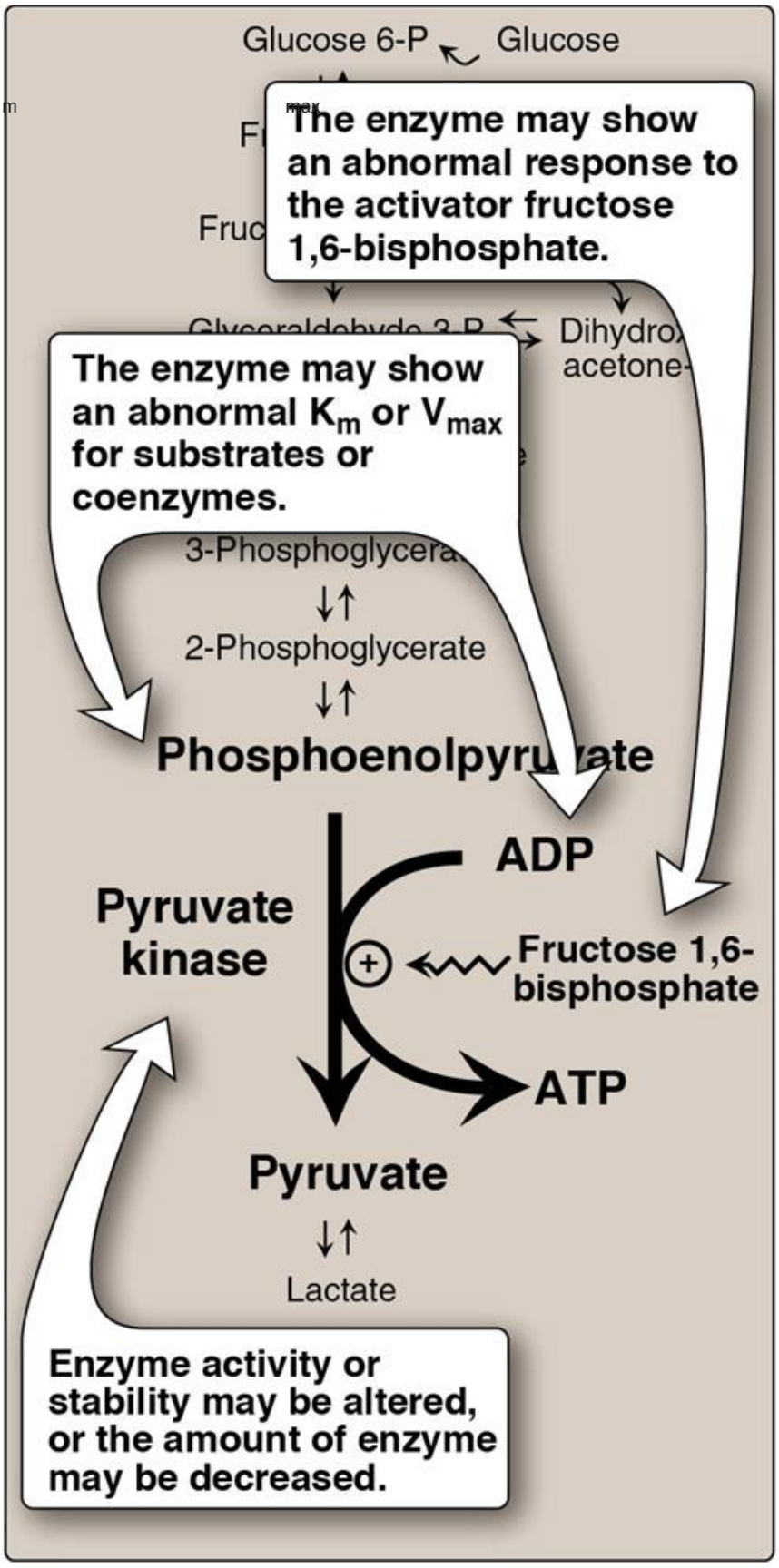
tivation of the enzyme.

P = phosphate; PP_i =

Pyruvate kinase deficiency

Because mature RBCs lack mitochondria, they are completely dependent on glycolysis for ATP production. ATP is required to meet the metabolic needs of RBCs and to fuel the ion pumps necessary for the maintenance of the flexible, biconcave shape that allows them to squeeze through narrow capillaries. The anemia observed in glycolytic enzyme deficiencies is a consequence of the reduced rate of glycolysis, leading to decreased ATP production by substrate-level phosphorylation. The resulting alterations in the RBC membrane lead to changes in cell shape and, ultimately, to phagocytosis by cells of the mononuclear phagocyte system, particularly splenic macrophages. The premature death and lysis of RBC result in mild-to-severe hemolytic anemia, with the severe form requiring regular transfusions. Among patients with rare genetic defects of glycolytic enzymes, the majority has a deficiency in PK. (Note: Liver PK is encoded by the same gene as the RBC isozyme. However, liver cells show no effect because they can synthesize more PK and can also generate ATP by oxidative phosphorylation.) Severity depends both on the degree of enzyme deficiency (generally 5% to 35% of normal levels) and on the extent to which RBC compensate by synthesizing increased levels of 2,3-BPG (see p. 32). Almost all individuals with PK deficiency have a mutant enzyme that shows altered kinetics or decreased stability (Fig. 8.20). Individuals heterozygous for PK deficiency have resistance to the most severe forms of malaria.

FIGURE 8.20



ase.

osphate.

The tissue-specific expression of PK in RBC and the liver results from the use of different start sites in transcription (see p. 473) of the gene that encodes the enzyme.

Pyruvate reduction to lactate

Lactate, formed from pyruvate by LDH, is the final product of anaerobic glycolysis in eukaryotic cells (Fig. 8.21). Reduction to lactate is the major fate for pyruvate in tissues that are poorly vascularized (e.g., the lens and cornea of the eye and the kidney medulla) or in RBC that lack mitochondria.

Lactate formation in muscle

In exercising skeletal muscle, NADH production (by glyceraldehyde 3-phosphate dehydrogenase and by the three NAD^+-linked dehydrogenases of the TCA cycle, see also Chapter 9) exceeds the oxidative capacity of the ETC. This results in an elevated $NADH/NAD^+$ ratio, favoring reduction of pyruvate to lactate by LDH. Therefore, during intense exercise, lactate accumulates in muscle, causing a drop in the intracellular pH, potentially resulting in cramps. Much of this lactate eventually diffuses into the bloodstream and can be used by the liver to make glucose.

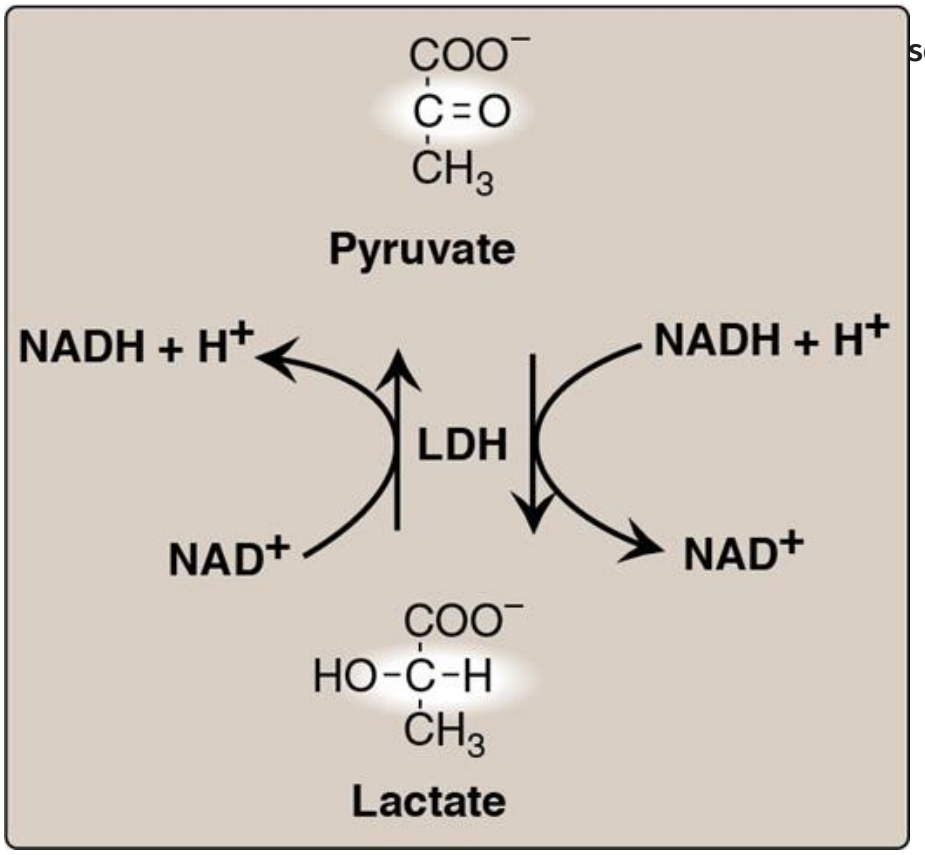
Lactate utilization

The direction of the LDH reaction depends on the relative intracellular concentrations of pyruvate and lactate and on the ratio of $NADH/NAD^+$. For example, in the liver and heart, this ratio is lower than in exercising muscle. Consequently, the liver and heart oxidize lactate (obtained from the blood) to pyruvate. In the liver, pyruvate is either converted to glucose by gluconeogenesis or converted to acetyl CoA that is oxidized in the TCA cycle. Heart muscle exclusively oxidizes lactate to carbon dioxide and water via the TCA cycle.

Lactic acidosis

Elevated concentrations of lactate in the plasma, termed lactic acidosis (a type of metabolic acidosis), occur when there is a collapse of the circulatory system, such as with myocardial infarction, pulmonary embolism, and uncontrolled hemorrhage, or when an individual is in shock. The failure to bring adequate amounts of O_2 to the tissues results in impaired oxidative phosphorylation and decreased ATP synthesis. To survive, the cells rely on anaerobic glycolysis for generating ATP, producing lactic acid as the end product. (Note: Production of even meager amounts of ATP may be lifesaving during the period required to reestablish adequate blood flow to the tissues.) The additional O_2 required to recover from a period when O_2 availability has been inadequate is termed the O_2 debt. (Note: The O_2 debt is often related to patient morbidity or mortality. In many clinical situations, measuring the blood levels of lactic acid allows the rapid, early detection of O_2 debt in patients and the monitoring of their recovery.)

FIGURE 8.21



se (LDH).

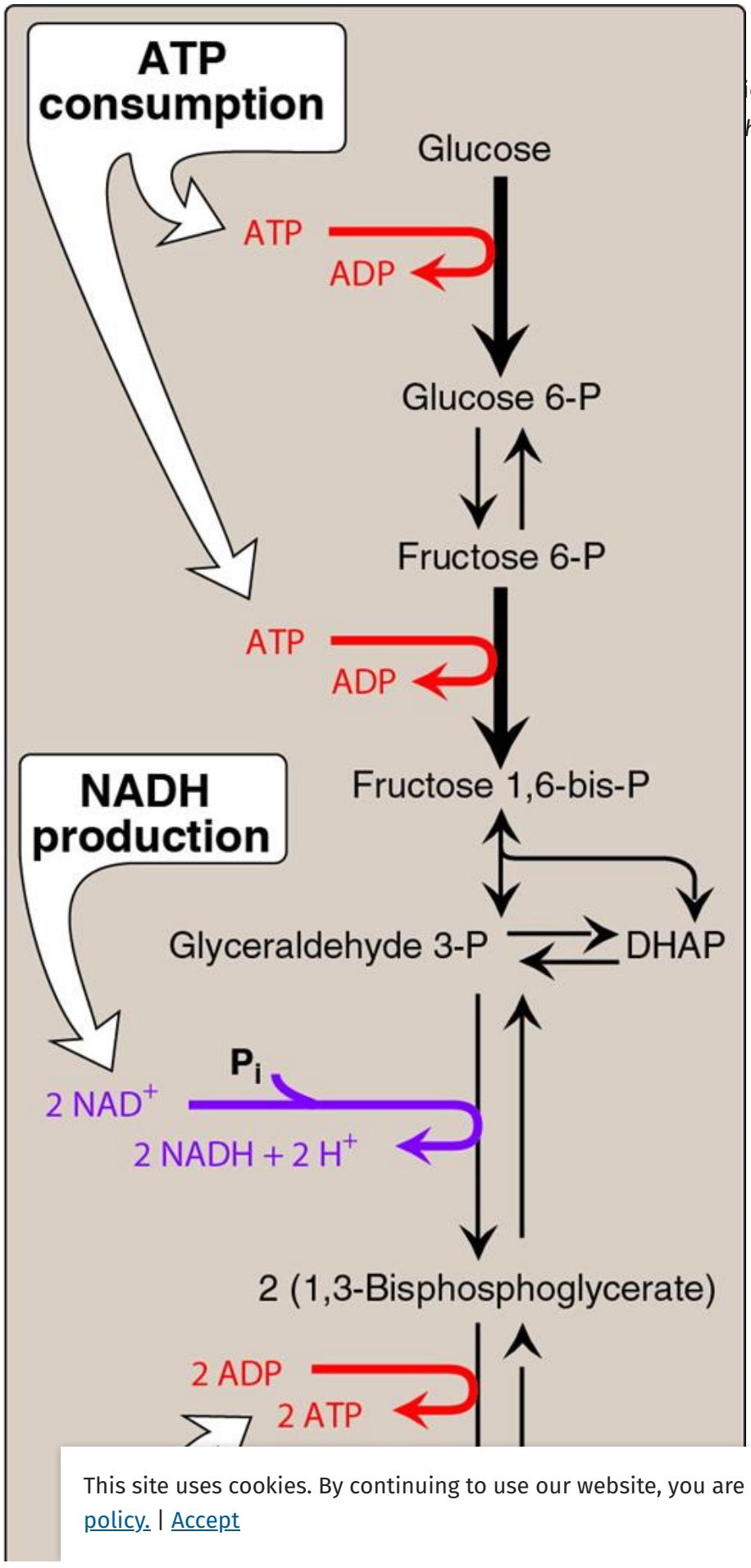
Energy yield from glycolysis

Despite the production of some ATP by substrate-level phosphorylation during glycolysis, the end product, pyruvate or lactate, still contains most of the energy originally contained in glucose. The TCA cycle is required to release that energy completely.

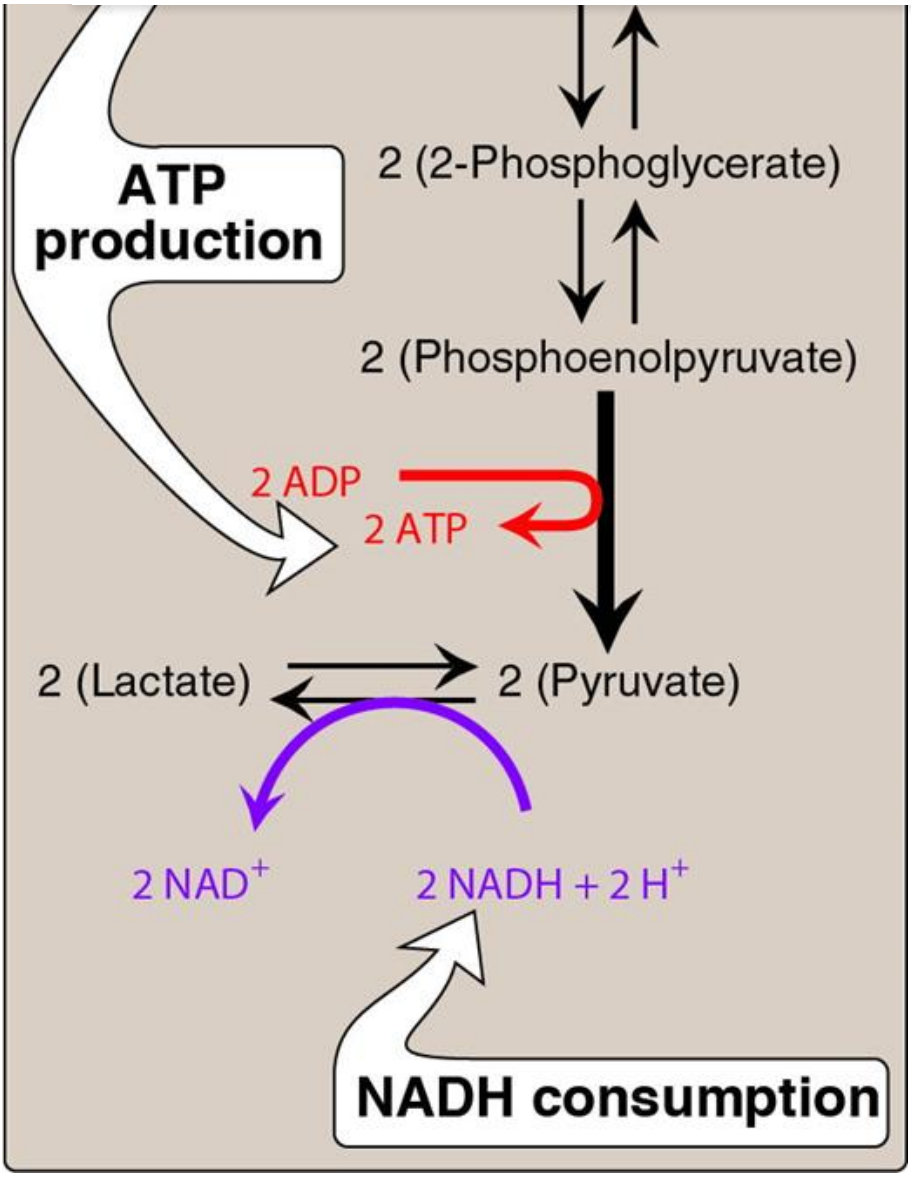
Anaerobic glycolysis

A net of two molecules of ATP are generated for each molecule of glucose converted to two molecules of lactate (Fig. 8.22). There is no net production or consumption of NADH.

FIGURE 8.22



ide adenine dinucleotide (NADH) are
hick arrows. DHAP = dihydroxyacetone



Aerobic glycolysis

The generation of ATP is the same as in anaerobic glycolysis (i.e., a net gain of two ATP per molecule of glucose). Two molecules of NADH are also produced per molecule of glucose. Ongoing aerobic glycolysis requires the oxidation of most of this NADH by the ETC, producing three ATP for each NADH molecule entering the chain (see p. 85). (Note: NADH cannot cross the inner mitochondrial membrane, and substrate shuttles are required.)

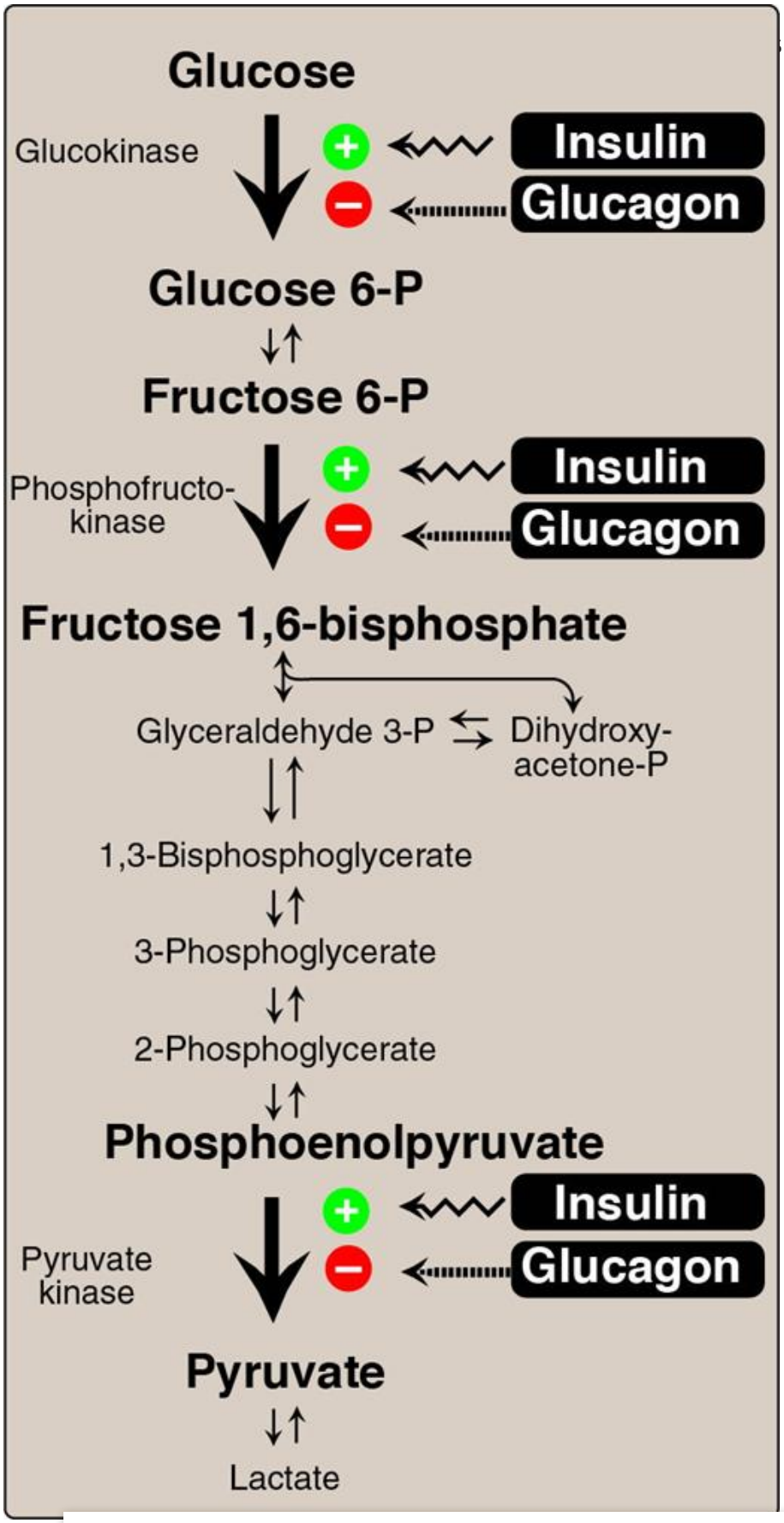
Hormonal Regulation

Listen

Regulation of the activity of the irreversible glycolytic enzymes by allosteric activation/inhibition or covalent phosphorylation/dephosphorylation is short term (i.e., the effects occur over minutes or hours). Superimposed on these effects on the activity of preexisting enzyme molecules are the long-term hormonal effects on the number of new enzyme molecules. These hormonal effects can result in 10- to 20-fold increases in enzyme synthesis that typically occur over hours to days.

Regular consumption of meals rich in carbohydrate or administration of insulin initiates an increase in the amount of glucokinase, PFK-1, and PK in the liver (Fig. 8.23). The change reflects an increase in gene transcription, resulting in increased enzyme synthesis. Increased availability of these three enzymes favors the conversion of glucose to pyruvate, a characteristic of the absorptive state. (Note: The transcriptional effects of insulin and carbohydrate [specifically glucose] are mediated by the transcription factors sterol regulatory element–binding protein-1c and carbohydrate response element–binding protein, respectively. These factors also regulate transcription of genes involved in fatty acid synthesis.) Conversely, gene expression of the three enzymes is decreased when plasma glucagon is high and insulin is low (e.g., as seen in fasting or diabetes).

FIGURE 8.23



of glycolysis in the liver.

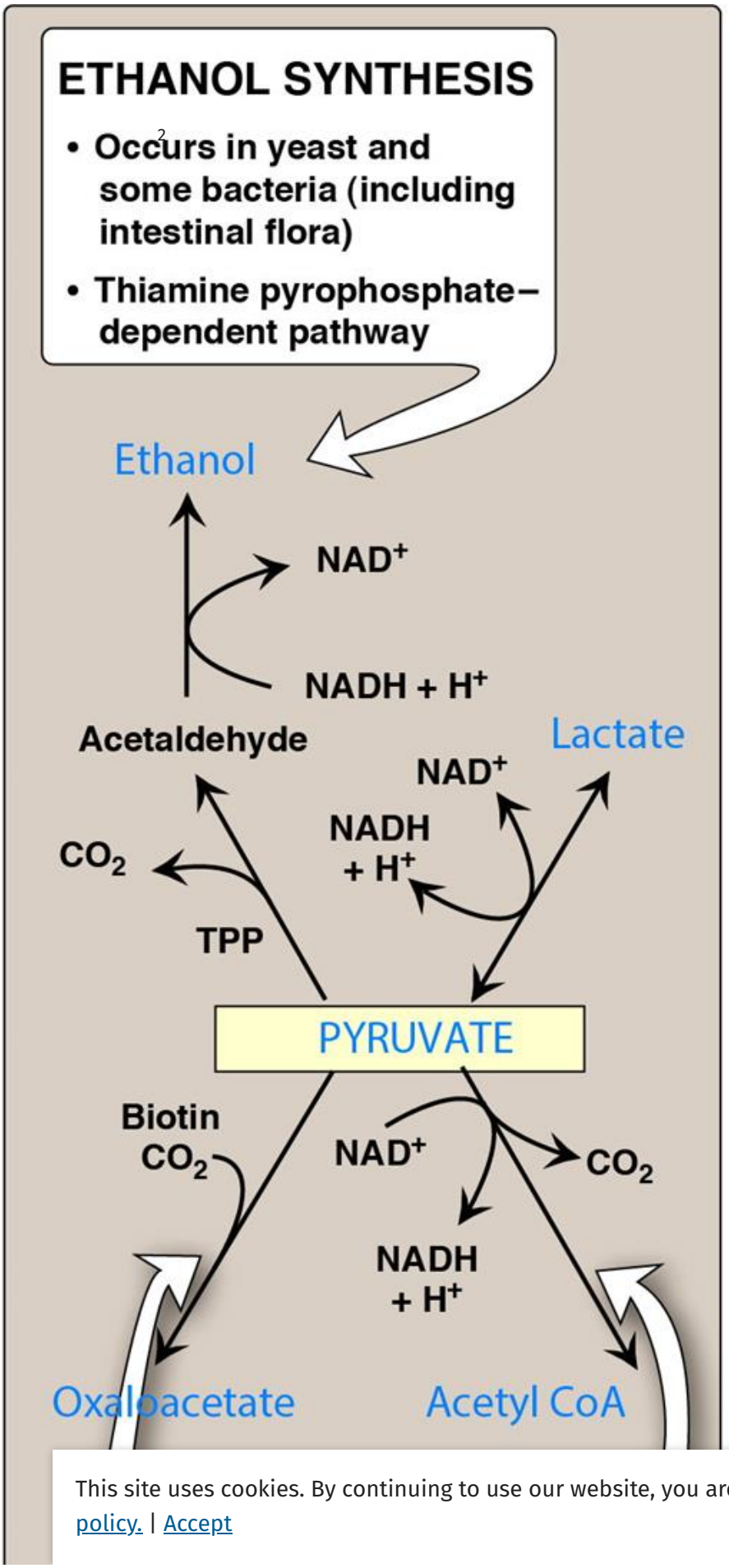
Alte

Pyruvate can be metabolized to products other than lactate.

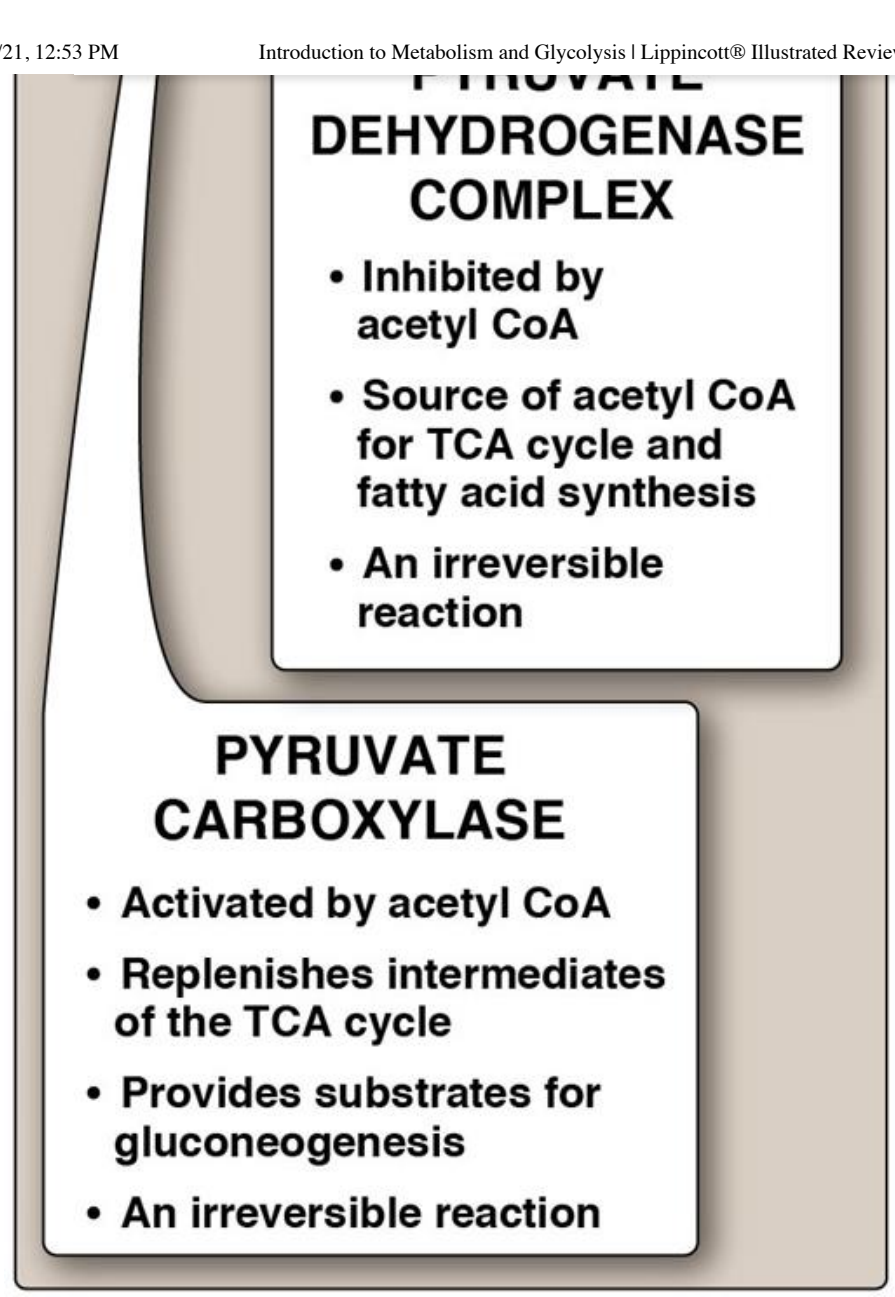
Oxidative decarboxylation to acetyl CoA

Oxidative decarboxylation of pyruvate by the PDHC is an important pathway in tissues with a high oxidative capacity such as cardiac muscle (Fig. 8.24). PDHC irreversibly converts pyruvate, the end product of aerobic glycolysis, into acetyl CoA, a TCA cycle substrate and the carbon source for fatty acid synthesis.

FIGURE 8.24



amide adenine dinucleotide; CoA =



Carboxylation to oxaloacetate

Carboxylation of pyruvate to oxaloacetate by pyruvate carboxylase is a biotin-dependent reaction (Fig. 8.24). This irreversible reaction is important because it replenishes the TCA cycle intermediate and provides substrate for gluconeogenesis.

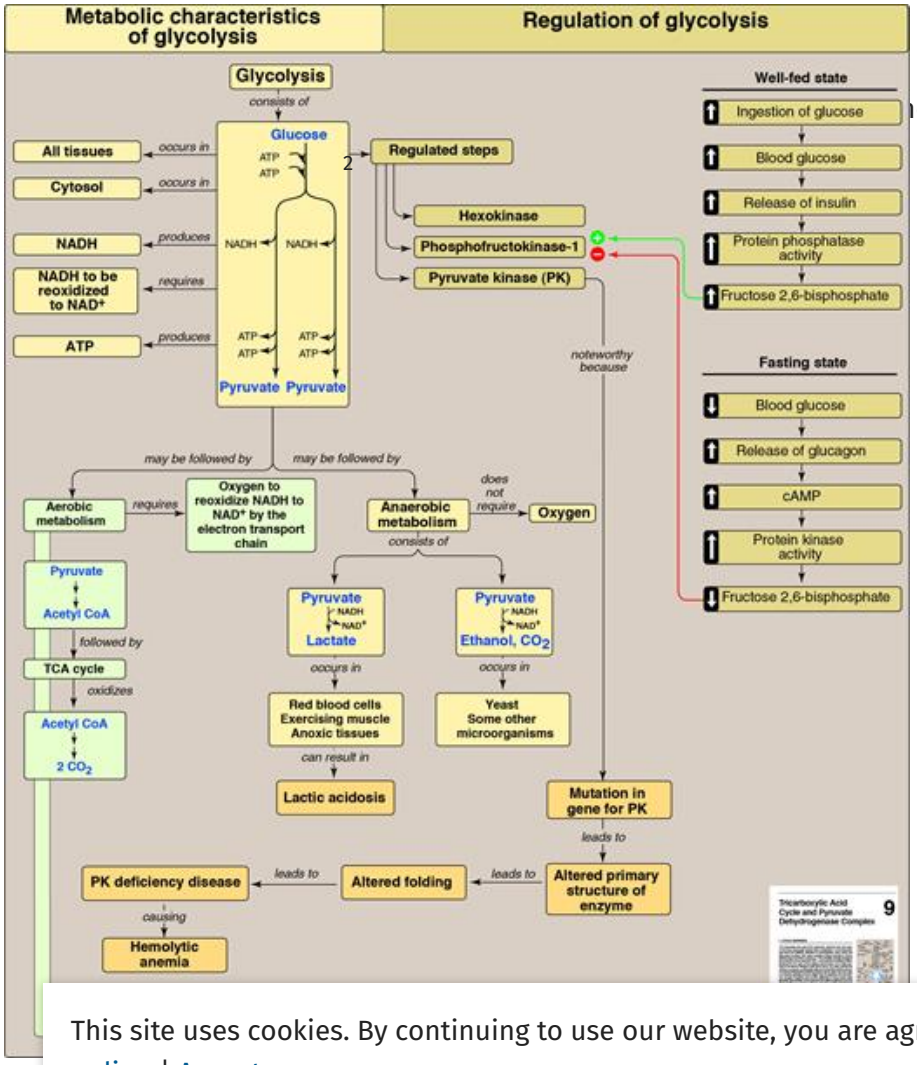
Reduction to ethanol (microorganisms)

The reduction of pyruvate to ethanol occurs by the two reactions summarized in Figure 8.24. The decarboxylation of pyruvate to acetaldehyde by thiamine-requiring pyruvate decarboxylase occurs in yeast and certain other microorganisms but not in humans.

Cha

- Most **pathways** can be classified as either **catabolic** (degrade complex molecules to a few simple products with **ATP production**) or **anabolic** (synthesize complex end products from simple precursors with **ATP hydrolysis**).
- **Intercellular** signaling provides for the integration of metabolism. The primary route of this communication is **chemical signaling** (e.g., by **hormones** or **neurotransmitters**).
- **Second messenger molecules** are regulated in response to GPCR and transduce a chemical signal to appropriate intracellular responders.
- **Adenylyl cyclase is** a cell membrane enzyme regulated by GPCR that catalyzes synthesizes **cyclic cAMP** in response to hormones **glucagon** and **epinephrine**.
- The cAMP produced activates **PKA**, which phosphorylates a variety of enzymes, on serine/threonine residues, causing their activation or deactivation.
- Phosphorylation is reversed by **phosphoprotein phosphatases.**
- **Aerobic glycolysis**, in which **pyruvate** is the end product, occurs in cells with mitochondria and an adequate supply of oxygen ([O_2], Fig. 8.25).

FIGURE 8.25



onophosphate; CoA = coenzyme A;

- **Anaerobic glycolysis**, in which **lactic acid** is the end product, occurs in cells that lack mitochondria and in cells deprived of sufficient O_2.
- Glucose is passively transported across membranes by **GLUTs** which have tissue-specific distributions.
- The oxidation of glucose to pyruvate (**glycolysis**, see Fig. 8.25) occurs through an **energy-investment** phase in which phosphorylated intermediates are synthesized at the expense of ATP and an **energy-generation** phase in which ATP is produced by **substrate-level phosphorylation**.
- **Hexokinase** has a **high affinity** (**low K_m**) and a **low maximal velocity** (**V_{max}**) for glucose and is inhibited by glucose 6-phosphate. **Glucokinase** has a high K_m and a high V_{max} for glucose. It is regulated indirectly by fructose 6-phosphate (inhibits) and glucose (activates) via **GKRP**.
- Glucose 6-phosphate is isomerized to **fructose 6-phosphate**, which is phosphorylated to **fructose 1,6-bisphosphate** by **PFK-1**. A total of **two ATPs** are used during this phase of glycolysis.
- Fructose 1,6-bisphosphate is cleaved to form two trioses that are further metabolized by the glycolytic pathway, forming pyruvate. During this phase, **four ATP** and **two NADHs** are produced per glucose molecule.
- The final step in pyruvate synthesis from **PEP** is catalyzed by **PK**. **PK deficiency** accounts for the majority of all inherited defects in glycolytic enzymes. Effects are restricted to **RBC** and present as mild-to-severe **chronic**, **hemolytic anemia**.
- Glycolytic gene **transcription** is enhanced by insulin and glucose.

Study Questions

Listen

Choose the ONE best answer.

8.1. Which of the following best describes the activity level and phosphorylation state of the listed hepatic enzymes in an individual who consumed a carbohydrate-rich meal about an hour ago? PFK-1 = phosphofructokinase-1; PFK-2 = phosphofructokinase-2; P = phosphorylated.

Choice	PFK-1		PFK-2		Pyruvate Kinase	
	Activity	P	Activity	P	Activity	P
A.	Low	No	Low	No	Low	No
B.	High	Yes	Low	Yes	Low	Yes
C.	High	No	High	No	High	No
D.	High	Yes	High	Yes	High	Yes

Correct answer = C. Immediately following a meal, blood glucose levels and hepatic uptake of glucose increase. The glucose is phosphorylated to glucose 6-phosphate and used in glycolysis. In response to the rise in blood glucose, the insulin/glucagon ratio increases. As a result, the kinase domain of PFK-2 is dephosphorylated and active. Its product, fructose 2,6-bisphosphate, allosterically activates PFK-1. (PFK-1 is not covalently regulated.) Active PFK-1 produces fructose 1,6-bisphosphate that is a feedforward activator of pyruvate kinase. Hepatic pyruvate kinase is covalently regulated, and the rise in insulin favors dephosphorylation and activation.

8.2. Which of the following statements is true for anabolic pathways only?

A. Their irreversible (nonequilibrium) reactions are regulated.

B. They are called cycles if they regenerate an intermediate.

C. They are convergent and generate a few simple products.

D. They are synthetic and require energy.

E. They typically require oxidized coenzymes.

Correct answer = D. Anabolic processes are synthetic and energy requiring (endergonic). Statements A and B apply to both anabolic and catabolic processes, whereas C and E apply only to catabolic processes.

8.3. Compared with the resting state, vigorously contracting skeletal muscle shows:

A. decreased AMP/ATP ratio.

B. decreased levels of fructose 2,6-bisphosphate.

C. decreased NADH/NAD^+ ratio.

D. increased oxygen availability.

E. increased reduction of pyruvate to lactate.

Correct answer = E. Vigorously contracting skeletal muscle shows an increase in the reduction of pyruvate to lactate compared with resting muscle. The levels of reduced nicotinamide adenine dinucleotide (NADH) increase and exceed the oxidative capacity of the electron transport chain. Consequently, the levels of adenosine monophosphate (AMP) increase. The concentration of fructose 2,6-bisphosphate is not a key regulatory factor in skeletal muscle.

8.4. Choose the correct statement. Glucose transport into:

A. brain cells is through active transport.

B. intestinal mucosal cells requires insulin.

C. liver cells involves a glucose transporter.

D. most cells is through simple diffusion.

Correct answer = C. Glucose uptake in the liver, brain, muscle, and adipose tissue is down a concentration gradient, and the transport is facilitated by tissue-specific glucose transporters (GLUTs). In adipose and muscle tissues, insulin is required for glucose uptake. Moving glucose against a concentration gradient requires energy and is seen with the sodium-dependent glucose cotransporter 1 (SGLT1) of intestinal mucosal cells. Except for some gasses, membrane transport into cells does not occur via simple diffusion. All glucose transport utilizes GLUT transport proteins.

8.5. Given that the K_m of glucokinase for glucose is 10 mM, whereas that of hexokinase is 0.1 mM, which isozyme will more closely approach V_{max} at the normal blood glucose concentration of 5 mM?

Correct answer = Hexokinase. K_m (Michaelis constant) is that substrate concentration that gives one half V_{max} (maximal velocity). When blood glucose concentration is 5 mM, hexokinase (K_m = 0.1 mM) will be saturated, but glucokinase (K_m = 10 mM) will not.

8.6. In patients with pertussis infection and whooping cough, $G\alpha_i$ is inhibited. How does this lead to a rise in cyclic adenosine monophosphate (cAMP)?

Correct answer = G proteins of the $G\alpha_i$ type inhibit adenylyl cyclase (AC) when their associated G protein–coupled receptor is bound by ligand. If $G\alpha_i$ is inhibited by pertussis toxin, AC production of cAMP is inapp